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Effects of reclamation effort on the recovery of ecosystem functions of a tropical degraded serpentinite dump site

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Abstract

Surface mining or other disturbing operations on ultramafic outcrops degrade soil conditions and decrease major ecosystem functions in those habitats. Human disturbances exacerbate the harsh conditions of some ultramafic soils, making recovery more difficult. Recovery of ecosystem functions of disturbed areas may vary from passive to active reclamation measures, depending on the starting situation, available resources and expected outcomes. In this study, three treatments (topsoil application, topsoil + non-compacted, and topsoil + non-compacted + organic amendment), representing a gradient of increasing reclamation effort, were tested for the reclamation of a tropical ultramafic Spolic Technosol in Sabah (Malaysia). Soil treatments were tested at the mesocosm-scale for 12 months, and drainage water fluxes, evolution of soil physicochemical parameters, soil microbial activities and spontaneous plant colonisation, diversity and growth were monitored along the duration of the experiment. The results show that application of topsoil improved the plant biomass and water filtration functions in the non-reclaimed sites, with no additional benefit from lesser compaction. The most intensive treatment (topsoil + non-compacted + organic amendment) gave the best improvements in nutrient cycling and biomass production functions, but with a reduction of water filtration function, due to an increase in soluble concentrations and cumulative fluxes of nickel. Introduced species were dominant in the vegetation colonizing the mesocosms, and therefore planting of selected native species will be necessary to create habitats for native biodiversity. This information will be useful to select a strategy for the recovery of degraded tropical serpentinite quarries and dump sites.

Keywords	amendments; ecosystem functions; multi-criteria assessment; soil reclamation; topsoil; tropical ultramafic areas
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Research Data Related to this Submission

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Data will be made available on request

Celestino Quintela-Sabarís, PhD
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Vandoeuvre, October 25th, 2018

Dear Dr. Ayuso,

I am enclosing herewith the reviewed version of the manuscript code GEXPLO_2018_215, which we submitted to consider for publication in the special issue Ni Laterite of *Journal of Geochemical Exploration*.

To produce this new version we have carefully addressed each of the comments provided by the reviewers. All the changes have been marked in yellow. We have modified all the figures to make them easier to understand, English usage has been reviewed and a new table has been included.

We hope you consider that this revised manuscript meets the requirements to be accepted for publication in *Journal of Geochemical Exploration*.

Thank you very much for your consideration.

Best regards,

Celestino Quintela-Sabarís, PhD

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Effects of reclamation effort on the recovery of ecosystem functions of a tropical degraded serpentinite dump site

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Abstract

Surface mining or other disturbing operations on ultramafic outcrops degrade soil conditions and decrease major ecosystem functions in those habitats. Human disturbances exacerbate the harsh conditions of some ultramafic soils, making recovery more difficult. Recovery of ecosystem functions of disturbed areas may vary from passive to active reclamation measures, depending on the starting situation, available resources and expected outcomes. In this study, three treatments (topsoil application, topsoil + non-compacted, and topsoil + non-compacted + organic amendment), representing a gradient of increasing reclamation effort, were tested for the reclamation of a tropical ultramafic Spolic Technosol in Sabah (Malaysia). Soil treatments were tested at the mesocosm-scale for 12 months, and drainage water fluxes, evolution of soil physicochemical parameters, soil microbial activities and spontaneous plant colonisation, diversity and growth were monitored along the duration of the experiment. The results show that application of topsoil improved the plant biomass and water filtration

functions in the non-reclaimed sites, with no additional benefit from lesser compaction. The most intensive treatment (topsoil + non-compacted + organic amendment) gave the best improvements in nutrient cycling and biomass production functions, but with a reduction of water filtration function, due to an increase in soluble concentrations and cumulative fluxes of nickel. Introduced species were dominant in the vegetation colonising the mesocosms, and therefore planting of selected native species will be necessary to create habitats for native biodiversity. This information will be useful to select a strategy for the recovery of degraded tropical serpentinite quarries and dump sites.

Keywords: amendments; ecosystem functions; multi-criteria assessment; soil reclamation; topsoil; tropical ultramafic areas.

1. Introduction

Ultramafic rocks have been exploited as ornamental stones, road-base aggregate, railway track ballast, and are mined to extract metals such as nickel (Ni), chromium (Cr) and cobalt (Co). Surface extractive operations including mining, quarrying, and public engineering (*i.e.* road construction) can degrade soil conditions via the compaction and the loss of topsoil, which in turn induces a decrease of major soil ecosystem services, such as limiting erosion, supporting vegetation, and creating a habitat for species (O'Dell and Claassen, 2009). These human disturbances exacerbate the already harsh conditions of many ultramafic areas, *e.g.* the nutrient imbalance and high concentrations of certain metals, making post-operation recovery even more challenging.

Ultramafic outcrops cover approximately 3% of the global terrestrial surface (Guillot and Hattori, 2013), and often host high levels of plant diversity especially in Southeast Asia (van der Ent et al., 2015). Soils derived from ultramafic bedrock are characterised by relatively high concentrations of certain trace elements, including Ni and Co, major cations imbalances with high magnesium to calcium (Mg:Ca) molar quotients) and nutrient deficiencies, especially potassium (K) and phosphorus (P) (Proctor 2003; Galey et al., 2017). Ultramafic soils differ substantial in their physical and chemical characteristics. Soils derived from serpentinite contains both primary minerals (chrysotile, antigorite, lizardite) and secondary minerals (smectites, magnetite, chlorite, talc) (Chardot et al., 2007), and is the most extreme in its chemical properties, with very high Mg:Ca molar quotients, high available Ni and high pH (van der Ent et al., 2017). Leptosols derived from these outcrops are eutrophic with high base saturation and CEC (Echevarria, 2018), and in Sabah hosts a specific and adapted vegetation that tolerates these geochemical peculiarities (van der Ent et al., 2015; 2016).

The recovery of vegetation on ultramafic degraded areas has thus multiple benefits: reduction of metal dispersion, decrease of soil erosion, and support of biodiversity (O'Dell and Claassen, 2009). For the vegetation to re-colonise disturbed ultramafic sites, it is necessary to re-establish the following soil functions: i) nutrient cycling to counteract the lack of essential nutrients; ii) water filtration to limit the dispersion of metals; iii) biodiversity hosting to provide habitat to endemic ultramafic flora; and iv) biomass production to prevent erosion and metal dispersion as well as to promote biodiversity. Reclamation techniques have different

effects on the targeted ecosystem functions. Mineral fertilisers have low effectiveness on revegetation success during recovery of ultramafic areas (O'Dell and Claassen, 2009). Moreover, their application may have undesirable side effects, such as increased mobility and leaching of trace elements (Raous et al., 2010). In contrast, topsoil re-application has been shown to be an effective method to revegetate ultramafic excavated substrates, provided that it is applied at a minimum depth of 30 cm, to allow for adequate rooting depth (O'Dell and Claassen, 2009). Moreover, in extremely anthropised landscapes there is often a lack of appropriate sources of plant germplasm and microbiota (Bradshaw, 2000; Rey Benayas et al., 2008). Topsoil can be used as an inoculum to provide seedbanks and microbiota to ensure the colonisation of disturbed soil (Bradshaw, 2000, 1997). However, this approach requires careful handling of topsoil, as well as stockpiling under appropriate conditions, and fast re-application of topsoil to ensure biological activity (Echevarria and Morel, 2015; Quintela-Sabaris et al., 2018). The use of organic wastes and industrial by-products may also be considered as an amendment for soil construction (Echevarria and Morel, 2015; O'Dell and Claassen, 2009; Séré et al., 2008). Along with nutrients, organic wastes provide soils with other positive effects, such as improvement of the soil structure, increasing cation exchange capacity (CEC), alleviation of hyper-alkaline pH associated with serpentinite minerals, and complexation of trace elements (Bradshaw, 1997).

Recent research to assess the reclamation success of post-operation areas focused on the evaluation of soil physicochemical parameters together with other compartments, such as soil microbiota and plants (Alvarenga et al., 2018) and relating this information with relevant ecosystem services (e.g. Burges et al., 2016). In this work on a degraded ultramafic area in Sabah (Malaysia) on the island of Borneo, various levels of intensity of the reclamation treatments were studied. In order to assess the quality of the four targeted ecosystem functions, we monitored drainage water fluxes, the evolution of soil physicochemical parameters, soil microbial activities and spontaneous plant colonisation diversity and growth at the mesocosm scale.

2. Material and Methods

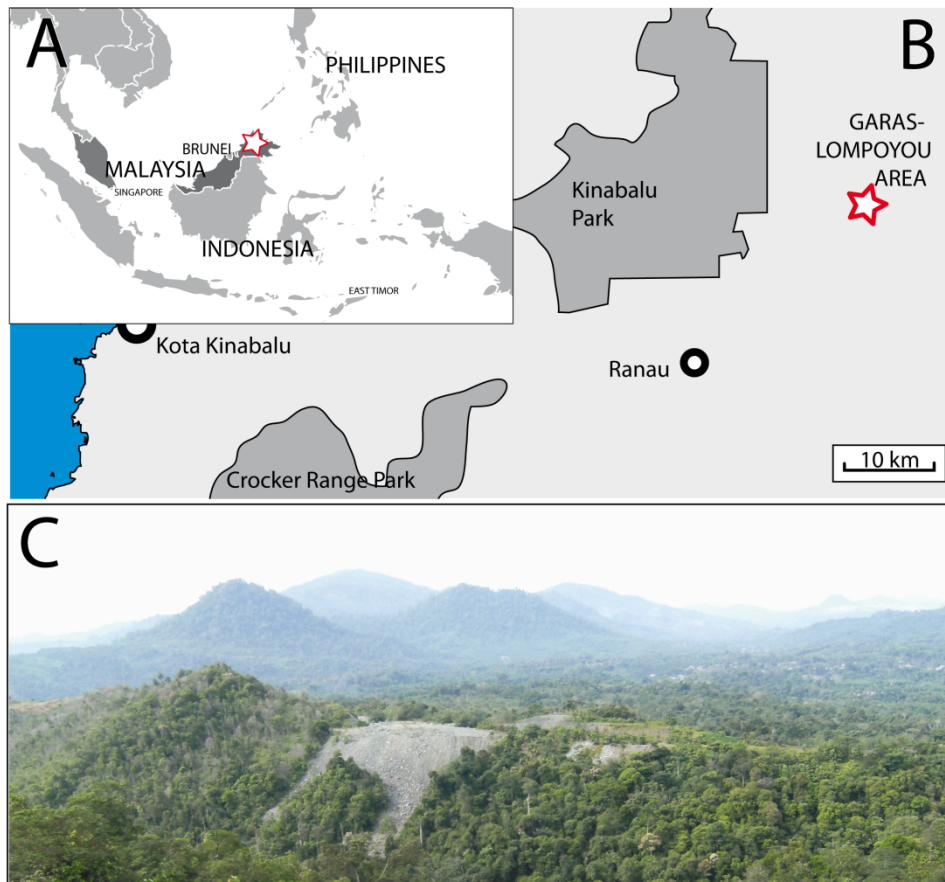
2.1 Studied site

Ultramafic outcrops cover 3500 km² in Sabah, in Malaysia, (Proctor et al., 1988; Repin, 1998) and 151 km² in Kinabalu Park (van der Ent et al., 2015). The study was undertaken at Garas, adjoining the main road from Ranau to Kota Marudu (latitude 6.088° N, longitude 116.798° E), which has a serpentinite outcrop with bare serpentinite soils (Hypereutric Leptosols Hypermagnesianic) (van der Ent et al., 2017). These soils have unweathered debris that is rich in clay minerals such as talc and smectite. The soil pH ranges from 6.5 in the soils rich in organic matter to pH 9.8 in the unweathered soil (van der Ent et al., 2017). Civil engineering activities for the enlargement of the road generated several dumpsites in 2008, that currently are poorly vegetated (Figure 1). The climate is tropical with a mean annual temperature of 23°C with low variation (less than 3°C) throughout the year. The annual rainfall is approximately 2,500 mm which is evenly distributed throughout the year. However, two less humid periods in February and August are typical (Aiba and Kitayama, 1999; Kitayama et al., 1998 and Malaysian meteorological department).

2.2 Parent material

Serpentine rock tailings (*i.e.* Spolic Technosols, IUSS Working Group WRB, 2015) were collected from one of the dump sites at Garas. In order to provide the experiment with a starting seed bank, ultramafic topsoil from a disturbed plot with secondary vegetation less than 100 m away from the dumpsite was collected. The plot had a pioneer plant community dominated by the fern *Pteridium esculentum* (Dennstaedtiaceae) and with the presence of different grasses (Poaceae), such as *Imperata cylindrica* and *Miscanthus floridulus*, and pioneer trees (including *Trema* sp. – Cannabaceae, and *Colona* sp. – Malvaceae). Several introduced species, that readily colonise disturbed areas in the region, were also present: *Mimosa pudica* (Fabaceae), *Lantana camara* (Verbenaceae), *Polygala sinensis* (Polygalaceae) and the invasive shrub *Chromolaena odorata* (Asteraceae).

The organic waste was chosen on the basis of its low price and high availability. Malaysia is the second largest world producer of palm-oil, and the cultivation and processing of oil palm (*Elaeis guineensis*) products generates an increasing quantity of liquid and solid organic wastes (more than 51 million tons in 2005, Kala et al., 2009). From the different types of oil palm organic wastes (Singh et al., 2010), we selected dry fronds (*i.e.* oil-palm leaves) as the organic amendment due to significant available stocks in the study area.



134

135 **Figure 1:** Studied area. **A**, regional map. **B**, local map. Dark grey areas mark Malaysia in the regional map, and
 136 protected areas of Sabah in the local map. Stars mark the area of study (Lompoyou – Garas) in the North of
 137 Borneo, East of Kinabalu Park. **C**, aspect of the study site in Garas (July 2016). Grey non-vegetated serpentinite
 138 rocks are prominent in the landscape.

139

140

141 2.3 Experimental setup

142 Sampled material (soils and oil-palm leaves) were transported to Monggis Substation
 143 (Kinabalu Park, coordinates latitude 6.200° N, longitude 116.752° E), where the experiment
 144 took place. Prior to experimental setup, serpentinite debris was sieved to remove rock
 145 fragments >25 cm. Oil-palm leaves were cut in pieces of ~2 cm diameter. Topsoil was
 146 thoroughly mixed and sieved <5 mm prior application. These materials were used for the
 147 construction of three different soil profiles (Fig. 2): *Compaction and topsoil*, (abbreviated as

COM) which consisted in a soil profile of 40 cm of compacted serpentinite debris –analogous to the current situation of serpentinite dump site at Garas– covered by ~5 cm of topsoil (see section 2.4); Non-compacted (abbreviated as NC), which consisted in a soil profile of ~40 cm of serpentinite debris with no compaction applied during filling of mesocosms, plus ~5 cm of topsoil; and Organic amendment (abbreviated as OM). This last treatment consisted in a soil profile of 20 cm of non-compacted serpentinite debris overlaid by ~20 cm of serpentinite debris mixed with 30% (volume:volume) oil-palm leaves (Table 1), plus ~5 cm of topsoil. At the bottom of these three different soil profiles, a 5-cm depth sand layer was placed in order to allow drainage of the mesocosms. A piece of geotextile was placed in between sand and serpentinite layers, to avoid particle eluviation. Each treatment was replicated four times. For each treatment, three replicates were studied in medium cylindrical mesocosms (surface 0.12 m², volume of 0.054 m³) and a fourth one was studied in a bigger cylindrical mesocosm (surface 0.20 m², volume 0.088 m³). A drainage system was installed in each mesocosm and connected to 5-L plastic bottles to allow the collection of drainage water. The mesocosms were placed outdoors on a flat surface fully exposed to sunlight and arranged in a randomised complete block design. The experiment started on July 13th 2016 and lasted for 12 months. No watering was applied to the mesocosms for the duration of the experiment, with rain the only water source.

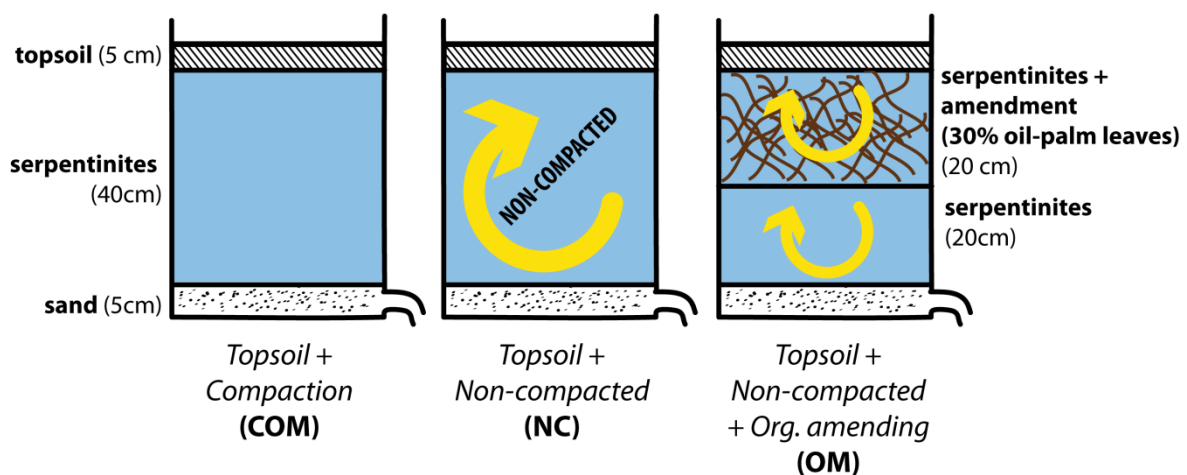


Figure 2: Soil profiles of the three soil treatments tested in mesocosms.

Element	Unit	Mean	SD
C _{org}	g kg ⁻¹	436	3.5
N	g kg ⁻¹	0.78	0.3
C:N		55.9	
Ca	g kg ⁻¹	4.93	0.37
Mg	g kg ⁻¹	2.84	0.36
Ca:Mg		1.74	
K	g kg ⁻¹	4.75	0.48
P	g kg ⁻¹	0.4	0.03
S	mg kg ⁻¹	730	53
Al	mg kg ⁻¹	179	12.6
Fe	mg kg ⁻¹	497	49.9
Mn	mg kg ⁻¹	217	14.5
Na	mg kg ⁻¹	< 162	-
Ni	mg kg ⁻¹	29.1	4.24
Cr	mg kg ⁻¹	9.07	1.19
Co	mg kg ⁻¹	2.78	0.21
Zn	mg kg ⁻¹	< 11	-

169

170 **Table 1:** Composition of oil-palm leaves which were used as organic amendment in OM treatment. Values
171 present the average, followed by SD (n = 3).

172

173 *2.4 Monitoring and sampling*

174 Spontaneous plant colonisation and plant cover was surveyed each two weeks for the duration
175 of the experiment. The monthly volume of drainage water was recorded. One sample (100 ml)
176 of drainage water was collected from each mesocosm every month.

177 At the end of the experiment (on July 2017) each mesocosm was processed as follows: shoots
178 were cut and collected separately for each species. Then, half of the mesocosm was removed
179 to expose the complete soil profile. Soil profile was refreshed until the centre of the pot. A
180 transparent plastic with a 5 × 5 cm grid was placed onto the soil profile to assess rooting depth
181 (Yilmaz et al., 2018). Three soil samples were collected at three depths (0–10 cm, 10–25 cm

and 20–45 cm). All root material from the entire mesocosm was collected as one bulk sample.

Plant material was thoroughly washed with tap water and then rinsed with deionised water.

Cleaned material was dried until constant weight. Fresh soil samples were sieved to <5 mm

and kept at 4° C until analyses.

2.5 Sample analyses

2.5.1 Drainage water

Upon collection, the pH was determined in the water samples. Following, the water samples were filtered (0.45 µm, Pall Acrodisc®) and preserved at 4°C until analysis. Water samples

were not acidified in order to measure concentrations of dissolved organic carbon (DOC).

However, due to delay in the site access, we were not able to perform this analysis in a decent

delay and just have estimates of DOC. The concentrations of Ca, Co, Cr, K, Mg, Na, Ni, P, S

and Zn were measured with Inductively Coupled Plasma- Atomic Emission Spectrometry

(ICP-AES, Liberty II, Varian Inc, Australia) and expressed as mg of element per L of drained

water.

2.5.2 Soils

Fresh soil samples were passed through a 5-mm sieve upon sampling. The activity of soil

enzymes alkaline phosphatase, arylsulphatase, β-glucosidase and urease, and the degradation

of fluorescein diacetate (FDA) were determined in soil fresh subsamples. The four enzymes

analysed are linked to the cycle of essential nutrients in the ecosystem: Carbon (β-

glucosidase), Nitrogen (urease). Phosphorus (alkaline phosphatase) and Sulphur

(arylsulphatase), whereas FDA degradation is generally considered a proxy for the general

hydrolytic activity of the soil (Bandick and Dick, 1999; Adam and Duncan, 2001).

Urease activity was determined using the colorimetric method described by Kandeler and

Gerber (1988), and expressed as µg NH₄-N g⁻¹ h⁻¹. Alkaline phosphatase, β-glucosidase and

arylsulphatase activities were determined using a microplate protocol and *p*-nitrophenol

(*p*NP) linked substrates (Dick et al., 2013). Before incubation, soils were treated with toluene

in order to release enzymes inside bacterial cells. Enzyme activity was determined from

colorimetric measurements of released *p*NP after incubating soil in a buffered substrate

solution (2 h, 37°C), and quantified by reference to calibration curves corresponding to *p*-

nitrophenol standards. Enzymatic activities are expressed in µg *p*-NP g⁻¹h⁻¹. Finally,

fluorescein diacetate (FDA) hydrolytic activity of the soil, an indicator of soil biological activity, was measured colorimetrically following the method of (Adam and Duncan, 2001). All values are expressed on an oven-dried soil basis.

Soil subsamples were air-dried and passed through a 2-mm sieve. The soil pH was measured in H₂O using a 1:5 (v/v) ratio. Cation exchange capacity (CEC) was determined colorimetrically after treatment of the soil with a solution of cobaltihexamine trichloride 0.05 N, following the standard method AFNOR NF-X 31-130 (Aran et al., 2008). Exchangeable concentrations of Ca⁺², Mg⁺² and K⁺ in the soil were determined on the filtered soil:cobaltihexamine extracts by ICP-AES (Liberty II, Varian). Total soil C and N measured with a CHNS combustion analyser (Vario Micro Cube, Elementar, Germany). Soil Ni availability was evaluated after extraction with DTPA-TEA (0.005 M DTPA with 0.01 M CaCl₂ and 0.1 M triethanolamine (TEA) at pH 7.3, 1:2 w/v, 2h shaking). Soil subsamples were ground in a ceramic mortar and 0.5 g of dry ground soil were digested in 2 mL of concentrated HNO₃ and 6 mL of concentrated HCl in a hot plate at 105°C. Final solutions were filtered (0.45 µm DigiFILTER, SCP science, Canada) and diluted to 50 mL with deionised water. Pseudo-total soil concentrations of Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P and Zn were measured with ICP-AES (Liberty II, Varian).

2.5.3 Plants

Shoot and root biomass are reported as dry weight (DW) from oven-dried plant samples. After DW was recorded, dry shoots and roots were finely ground using a ball mill. Subsamples (0.5 g) of dry and ground tissue were digested at 95°C in 2.5 mL of concentrated HNO₃ and 5 mL of H₂O₂ (30%). The final solutions were filtered (0.45 µm DigiFILTER) and diluted to 25 mL with deionised water. Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni and P concentrations in roots and shoots were measured with ICP-AES (Liberty II, Varian) and expressed in mg g⁻¹ DW plant material. C and N were measured in dry ground roots and shoots using a CHNS analyser (Vario Micro Cube, Elementar, Germany).

2.6 Data analysis

The monthly volume of drained water was divided by surface of mesocosm in order to compare mesocosms of different size. Monthly concentrations of Ca, Cr, K, Mg, Na, Ni, P, S and Zn in drainage water were multiplied by monthly volume of drained water (L m^{-2}) to obtain the monthly elemental flux (g m^{-2}). Differences between treatments in monthly elemental flux were assessed by means of Principal Components Analysis (PCA). Cumulative fluxes of Co, Cr and Ni (main trace elements in ultramafic soils) at harvest were assessed by one-way ANOVA analyses (factor *treatment*).

A PCA analysis was performed on the soil dataset including pH, CEC, soil enzyme activities and total, exchangeable and DTPA-extractable concentrations of several elements. Phosphorus was excluded from the analysis because all samples collected at 10–20 and 20–40 cm depth had P concentrations below quantification limit (approx. 100 mg kg^{-1}). Selected soil variables were further explored by blocked two-way ANOVAs (factors *treatment* and *depth*). Plant diversity in each mesocosm was calculated using Shannon's diversity index (H'). This index was computed with the following formula: $H' = -\sum p_i \ln(p_i)$. Where i corresponds to each species in the vessel and p_i is the proportional abundance of species i . Differences in plant species composition between treatments were assessed by 2D Non-Metric Multidimensional Scaling (NMDS). In both cases, shoot biomass data was used as a proxy for the abundance of each species. H' was computed with function *diversity*, and NMDS was computed using function *metaMDS*, both of them from package *vegan* for R. The time until the detection of the first plant growing on each mesocosm was registered. Time data were used to assess the effects of treatment on spontaneous plant colonisation. Between-treatment differences in time to the first colonising plant, in plant diversity and root and shoot biomass at harvest were assessed by blocked one-way ANOVA (factor *treatment*), whereas differences in vertical root distribution at harvest were assessed by blocked two-way ANOVA (factors *depth* and *treatment*). A PCA analysis was performed on plant chemical data (i.e. total root and shoot concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, N, Ni and P). These data were further explored by blocked two-way ANOVA (factors *fraction* –root vs shoot– and *treatment*). PCAs and ANOVA were performed in SPSS (v. 15, SPSS Inc., Chicago, IL, USA).

272 A multi-criteria assessment of treatment effects on four ecosystem functions (biomass
 273 production, nutrient cycling, water filtration and plant biodiversity habitat) has been
 274 developed. For that, several of the measured variables have been selected and grouped as
 275 indicators for each function (Table 2). The values of each indicator were ranked in four
 276 classes (0, +, ++, +++) and computed an average rank for each treatment and ecosystem
 277 function. Trace elements and nutrient concentrations in soils were chosen as explanatory
 278 variables to aid in the interpretation of treatment results.

279

Functions	Indicators
Biomass production	Aerial dry biomass
	Root dry biomass
	Plant cover
Nutrient cycling	Total cumulative fluxes of K, Ca, Na, P, S
	Nutrient shoot concentrations (C, N, P, K, S, Ca)
	Nutrient root concentrations (C, N, P, K, S, Ca)
	Enzyme activities and FDA (at 20 cm)
	Root abundance at 20 cm depth
Water filtration	Total cumulative fluxes of Ni, Cr, Co, Zn
	Maximal concentration of Ni, Cr, Co, Zn
	Exceedance of critical limits
Plant biodiversity habitat	Number of species
	Shannon's Index
	Presence of introduced species (N of species and % of total shoot biomass)
Explanatory variables	Nutrient and metal (total and available) concentrations in soils

280

281 **Table 2:** Selected indicators for the determination of four ecosystem functions in the experiment. Explanatory
 282 variables for the treatment results are presented in last row.

3. Results

3.1 Evolution of drainage water

Accumulated precipitation for the 12 months duration of the experiment nearly reached 3000 mm. The accumulated volume of drained water from the mesocosms was ~2073 mm per mesocosm (± 393 mm), with no difference between the treatments (one-way ANOVA, $p = 0.799$). PCA analysis of the drainage water yielded three Principal Components (PCs) that explained 71% of total variance of drainage water composition. PC1 (30% of variance) was mainly determined by fluxes of Ca, Na and S; whereas PC2 (28% of variance) was related to fluxes of K, Mg and Ni. Cr and P reflect on PC3 (13% of variance) (Fig. 3A). Considering first two PCs, each group of variables had different behaviour: Ca, Na and S fluxes (PC1) were time dependent (*i.e.* a higher flux at the beginning of the experiment and fluxes reduced with time, Fig. 3B). Conversely, K, Mg and Ni fluxes were independent of time but more related to the volume of drainage water (*i.e.* linked with the volumes of drainage water, Figure 3B). The position of samples on the two PCs shows that COM and NC treatments follow similar trends: they vary along PC1 but, for almost all samples, they stay in negative values of PC2 (*i.e.* lower fluxes of K, Mg and Ni). OM samples have similar variation along PC1, but have positive values in PC2, which indicates increased flux of K, Mg and Ni (Fig. 3). All the treatments had maximum values in PC1 during first three months, coinciding with the highest concentrations of Ca, Na and S in the drainage water.

The three treatments had contrasting effects on the soluble concentrations of Cr and Ni. Co concentrations were always below the limit of quantification (0.002 mg L^{-1}). In the case of Cr, OM treatment reduced soluble concentrations from 0.01 mg L^{-1} (treatment COM) to 0.005 mg L^{-1} (treatment OM, Fig. 4A). However, all water samples had Cr concentrations lower than the EU threshold for Cr in drinking water (*i.e.* 0.05 mg L^{-1} , Council Directive 98/83/EC). The organic amendment has the opposite effect on Ni: soluble concentrations of this element increased in the OM treatment (0.038 mg L^{-1}) with respect to other two treatments (0.007 mg L^{-1} , Fig. 4A). Thus, most of the water samples from OM treatment exceeded the EU threshold for Ni in drinking water (*i.e.* 0.02 mg L^{-1} , Council Directive 98/83/EC).

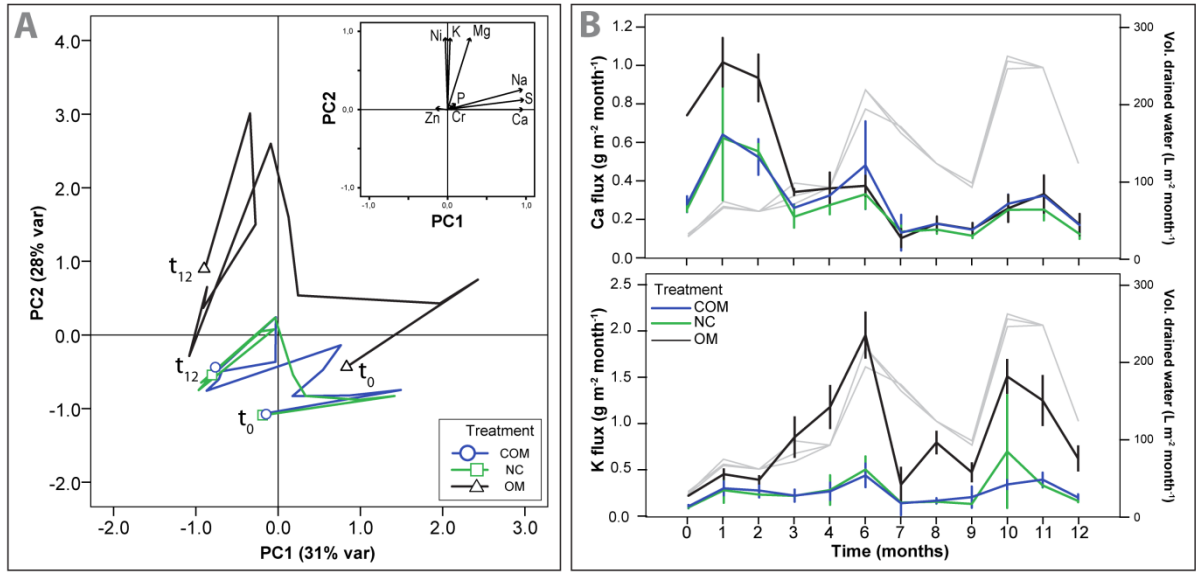


Figure 3: A PCA analysis of elemental flux in drainage water. Different lines present the evolution of monthly fluxes for each treatment. Circles, squares and triangles mark elemental flux at $t = 0$ (July 2016) and at $t = 12$ (July 2017) for each treatment. Contribution of each measured variable to PCs is presented in the inset. **B** Evolution of monthly elemental flux for Ca (top) and K (bottom) along the experiment ($\text{g m}^{-2} \text{ month}^{-1}$). Different colours correspond to average values for each treatment ($\pm \text{SD}$): blue, COM; green, NC; black, OM. Grey lines present the monthly volumes of drained water ($\text{L m}^{-2} \text{ month}^{-1}$), averaged for each treatment.

As a result of the variation in soluble concentrations of Cr and Ni, a variation in cumulative flux of these elements was observed. In the case of Cr, the OM treatment reduced accumulated flux to about half of the other treatments (0.07 g m^{-2} in OM vs. 0.15 g m^{-2} in COM and NC, Fig. 4B). In the case of Ni, there was an opposite trend: Ni accumulated flux increased up to nearly 0.06 g m^{-2} in the OM treatment, whereas it was approximately 0.01 g m^{-2} in other two treatments (Fig. 4B).

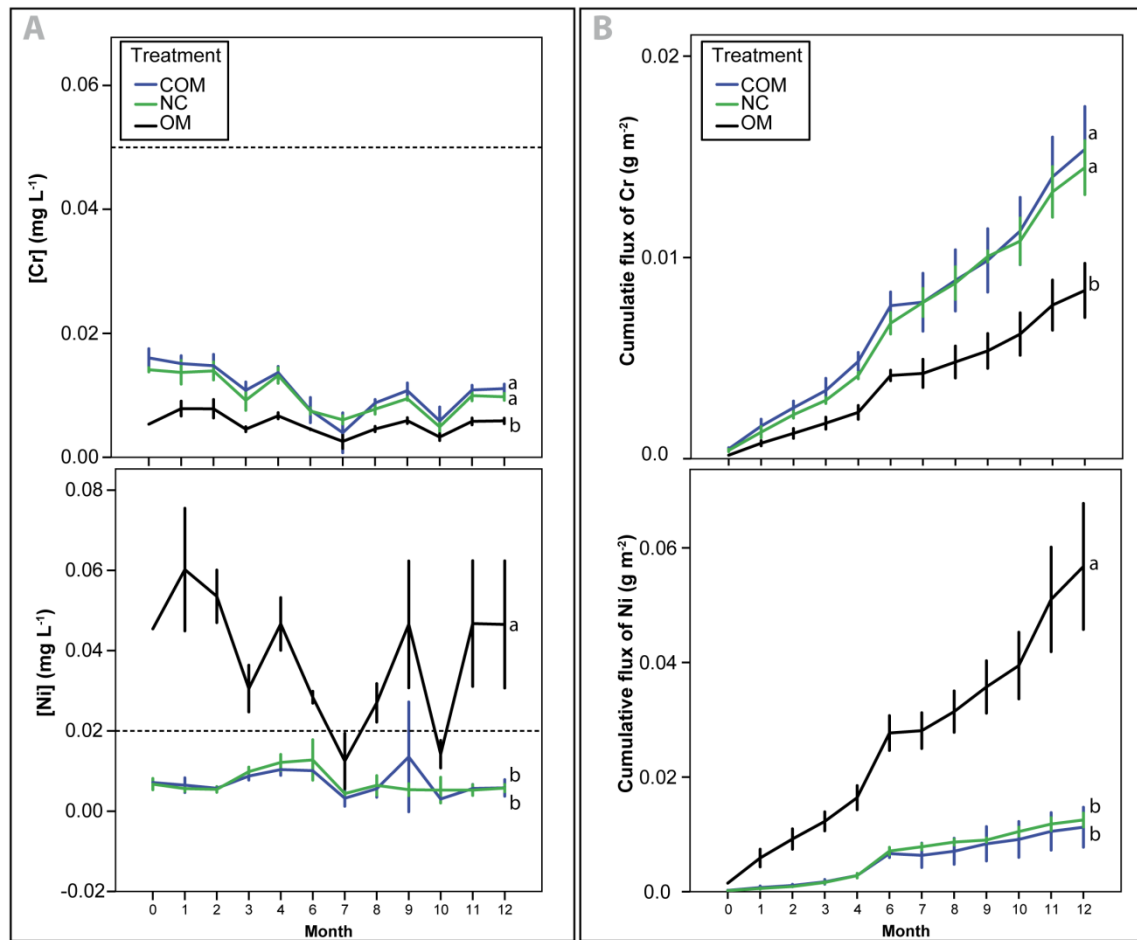


Figure 4: Variation in Cr and Ni soluble concentrations (A) and cumulative element flux (B). Dashed horizontal lines in 5A mark the EU threshold for Cr and Ni in drinking water. Different letters mark significant differences ($p < 0.05$) between *treatments* (posthoc analysis with Bonferroni correction). Different colours correspond to average values for each treatment ($\pm$ SD): blue, COM; green, NC; black, OM.

3.2 Soil parameters

During experiment set-up, we applied a 5-cm thick layer of topsoil on the three tested treatments. Water infiltration and root development contributed to the mixing of topsoil with the underneath serpentinite debris. Thus, at harvest, we observed in all treatments a top 10-cm thick reddish-coloured layer contrasting with the deeper grey-blue soil layers.

A PCA was performed on soil chemical and biological parameters. Three PCs were inferred, explaining 92% of total variance in the soil parameters. The PC1 alone accounted for most of the variance (85%). Variables with positive loadings in this PC included all measures of

biological activity, **pseudo**total Al, Ca, K, C and N concentrations, CEC and exchangeable Ca and Ni, and DTPA-extractable concentrations of Co and Ni. In contrast, pH, total concentrations of Fe, Mg and trace elements (Co, Cu, Mn, Ni and Zn) and exchangeable Mg had negative loadings on PC1. PC2, which explained 5% of variance only, was contributed by total Cr concentration and exchangeable K (Figure 5A). Projection of soil samples on two first PCs showed a clear differentiation between soil depths. Samples of the upper part of the soil profile (which correspond to contact between topsoil – similarly in the three treatments– and serpentinite debris) were placed in positive values for PC1, and near 0 for PC2 (Fig. 5B). Thus, these samples had the best chemical and biological fertility parameters among all the analysed soils (*i.e.* higher CEC, C, N, Ca and K concentrations, higher enzyme activities). In contrast, samples of the middle (10-25 cm depth) and the bottom (25-45 cm depth) of the soil profile in treatments COM and NC were plotted in the most negative values of PC1 (*i.e.* higher pH, exchangeable Mg and total concentrations of Mg and trace elements, Fig 5). Interestingly, samples of 10-25 cm – where oil-palm leaves were applied – and 25-45 cm of OM treatment were placed in less-negative values of PC1 than samples from other treatments at same depths, and also had the most positive values in PC2 (*i.e.* better chemical fertility parameters compared to other two treatments and higher Cr and exchangeable K).

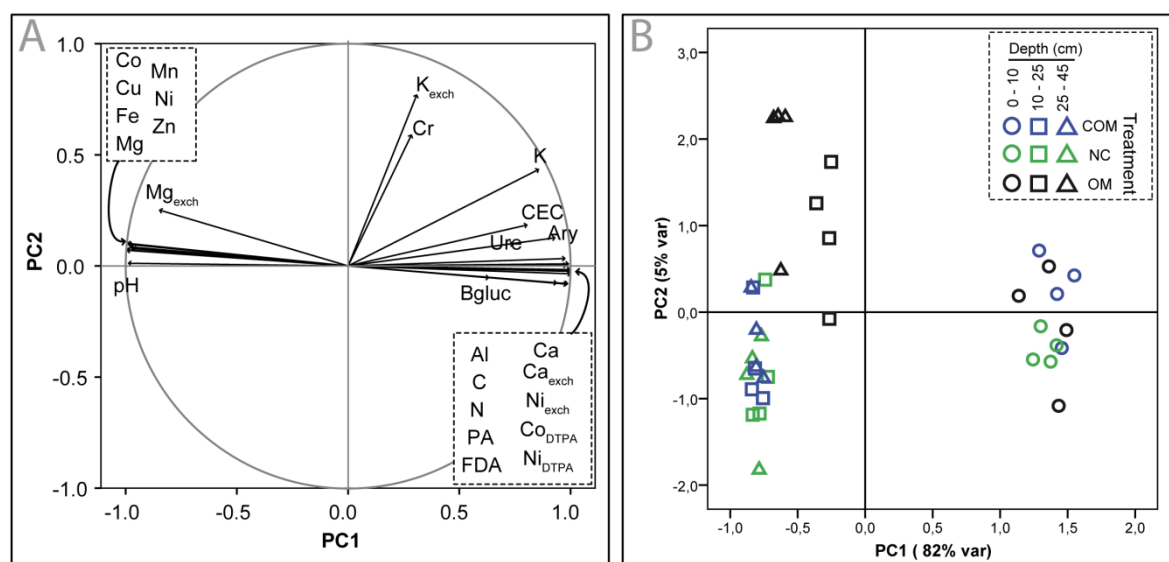
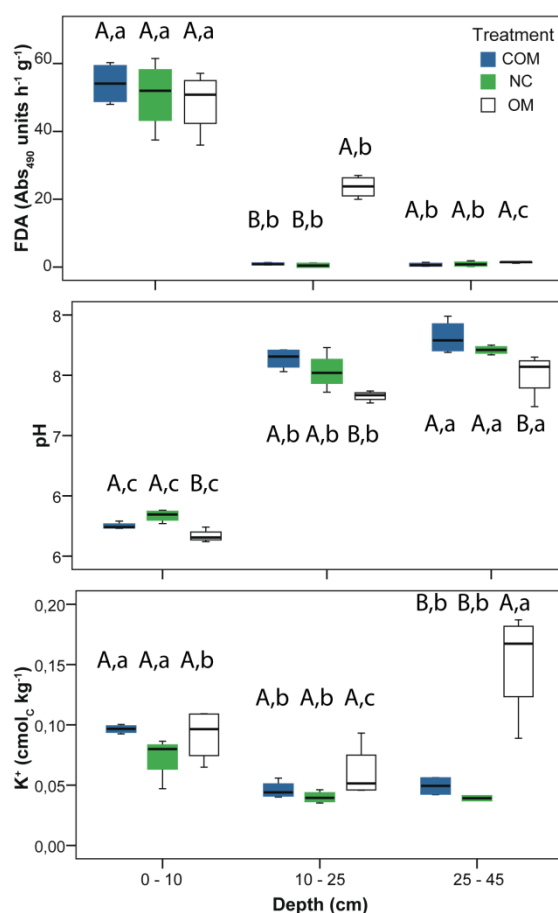


Figure 5: PCA of soil parameters. (A) Loadings of each soil variable in two first Principal Components (PCs). (B) Projection of soil samples (each symbol indicates one sample) on first two PCs. Different colours correspond

to each treatment (blue, COM; green, NC; black, OM), whereas different symbols mark depth (circles, 0-10 cm; squares, 10-25 cm; triangles, 25-45 cm). Concentrations of different elements are abbreviated as their chemical symbols (plus _{DTPA} and _{exch} sub indices in DTPA-extractable and exchangeable concentrations, respectively) in (A), whereas soil enzymes were abbreviated as follows: Ary, arylsulphatase; Bgluc, β -glucosidase; FDA, fluorescein diacetate degradation; PA, alkaline phosphatase; Ure, urease.

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The patterns unveiled by soil PCA were further explored by ANOVAs. Three soil variables related to PCs (FDA degradation, positive contribution to PC1; pH, negative contribution to PC1; and K exchangeable concentration, positive contribution to PC2; Fig. 6) were compared. In the three cases, COM and NC had similar values, whereas treatment OM had a different response. Organic matter addition significantly increased FDA degradation at intermediate depth; reduced pH at three depths considered and increased exchangeable K concentration at 25-45 cm depth in the profile (Fig. 6).



375

Figure 6: Differences in soil parameters related to two first Principal Components of soils PCA (FDA degradation, pH and exchangeable K concentration) between different treatments and soil depths. Each treatment is indicated by a different colour (blue, COM; green, NC; black, OM) whereas depth is indicated along the horizontal axis. In each plot, different capital letters indicate significant differences between *treatments* within each *depth* and different lower case letters denote significant differences across all *depths* within each *treatment*.

3.3 Plant diversity and other plant parameters

A total of 17 different plant species colonised the 12 mesocosms. The number of species per mesocosm ranked from 3 to 14. Eight species were present in just one mesocosm. Two introduced species were present in the majority of pots: *Chromolaena odorata* (12 pots) and *Polygala sinensis* (10 pots) (Table 3). Plant communities were similar in the three treatments, as indicated by the partially-overlying areas in the NMDS graph (Fig. S1, supplementary material).

Species code	Species name	Plant family	N of mesocosms
CHOD *	<i>Chromolaena odorata</i>	Asteraceae	12
POSI *	<i>Polygala sinensis</i>	Polygalaceae	11
SCSP	<i>Schoenoplectus sp.</i>	Cyperaceae	10
G#01	Grass01	Poaceae	7
PASP	<i>Paspalum sp.</i>	Poaceae	6
NEGI	<i>Neonauclea gigantea</i>	Rubiaceae	4
CASP	<i>Carex sp.</i>	Cyperaceae	3
PACO *	<i>Paspalum conjugatum</i>	Poaceae	3
BLBA	<i>Blumea balsamifera</i>	Asteraceae	2
CYSP	<i>Cyperus sp.</i>	Cyperaceae	1
TRSP	<i>Trema sp.</i>	Cannabaceae	1
AS#01	Asteraceae 01	Asteraceae	1
F#01	Herbaceous Forb 01	? (Dicot)	1
F#02	Herbaceous Forb 02	? (Dicot)	1
S#01	Shrub 01	? (Dicot)	1
T#01	Tree01	? (Dicot)	1
T#02	Tree02	? (Dicot)	1

Table 3: List of species colonising ultramafic mesocosms. For each species the more precise identification is provided. Asterisks (*) next to species code indicate alien species. Last column indicates the number of mesocosms where each species was found.

With regard to the plant diversity and biomass, significant differences were only found in shoot biomass (Table 4). For this parameter, the OM treatment yielded higher shoot biomass (average of 33.6 g per mesocosm) than any of the other two treatments, and nearly triplicated biomass values compared to COM. Shoot: root ratio differed among treatments. Treatment OM had the highest value (2.56), whereas the lower was in treatment COM (1.54). There were no significant differences in plant cover between treatments. Cover values ranked from 66.2% (average for treatment NC) and 91.4% (average for treatment OM).

Treatment	N sp	Shannon H'	Shoot biomass (g)	Root biomass (g)	Shoot:Root ratio	Plant cover (%)
COM	4.5 ± 1.3	1.03 ± 0.37	13.9 ^b ± 3.0	9.2 ± 2.0	1.54 ± 0.29 ^b	77.9 ± 11.5
NC	4.7 ± 1.0	0.82 ± 0.45	19.4 ^{ab} ± 7.8	12.4 ± 5.2	1.58 ± 0.28 ^{ab}	66.2 ± 23.3
OM	7.2 ± 4.6	1.04 ± 0.28	33.6 ^a ± 14.6	13.6 ± 6.5	2.56 ± 0.57 ^a	91.4 ± 6.5
Grand mean (12 mesocosms)	5.5	0.96	22.3	11.7	1.89	78.5

Table 4: Summary of plant diversity and biomass in soil mesocosms, and plant cover at harvest. Mean and SD values are presented for each variable. Different superscript letters indicate significant differences between treatments (p < 0.05).

The number of plants growing in the mesocosm varied in different ways between the treatments, although there was no significant difference between the treatments at harvest (Fig. 7). However, there were significant differences in the time to the appearance of the first colonising plant. In COM, colonisation was faster than other two treatments, average time for the first coloniser was 45 days. The number of plants peaked at around 200 days from the start of experiment. However, plant mortality (that affected especially the seedlings) reduced progressively the number of individuals, and around day 300 there were (on average) fewer than 25 plants per mesocosm. The first coloniser in treatment NC appeared (on average) at day 77. Plant colonisation was low along all the experiment, but during the last month the number of plants strongly increased to mean values higher than 75 plants per mesocosm. The plant colonisation in treatment OM was delayed until day 108 and followed a similar pattern

as treatment NC during first half of the experiment. Around day 250 there was an important increase in the number of plants per mesocosm. After a strong decrease in the number of plants around day 300, there was a fast increase until values higher than 50 plants per mesocosm.

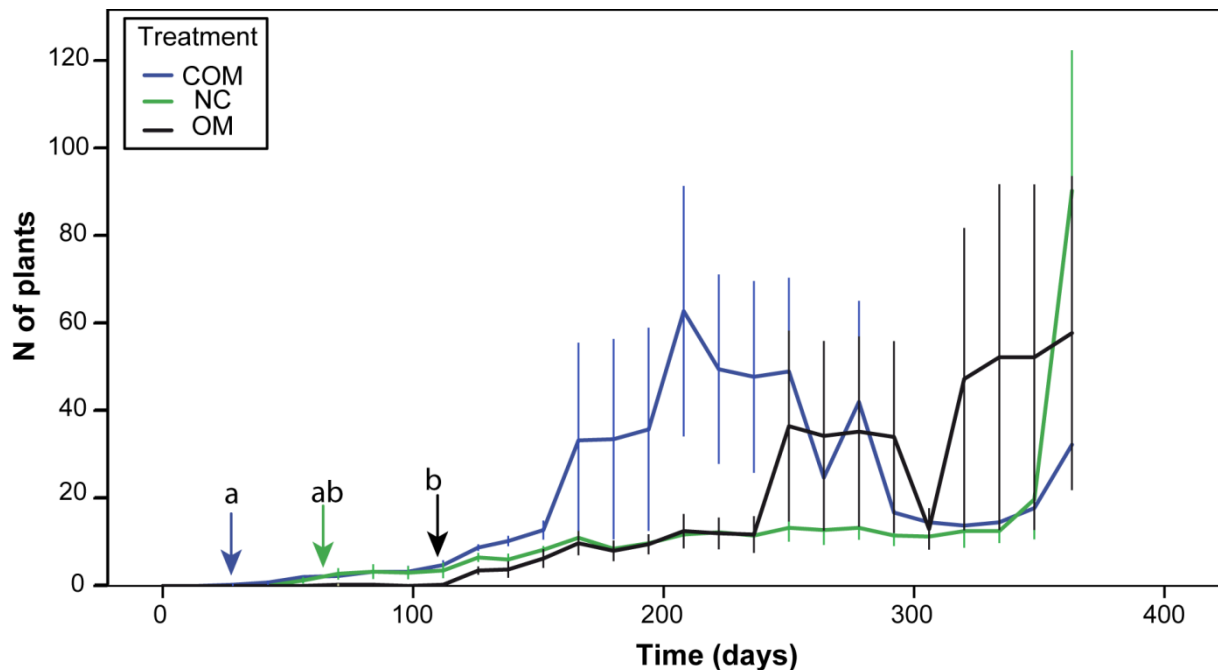


Figure 7: Evolution of plant colonisation of mesocosms. Lines indicate the average number of plants ($\pm$ SD) per treatment (blue, COM; green, NC; black, OM) at each number of days from the beginning of the experiment. Vertical arrows mark for each treatment the average number of days to the detection of the first coloniser plant in a mesocosm, with different superscripts marking significant differences ($p < 0.05$) between treatments.

Despite of the lack of differences in root biomass, we found significant differences in vertical root distribution along soil profile. The three treatments had similar root abundances down to 15 cm deep. At increasing depths, root abundance decreased for treatments COM and NC whereas in OM treatment root abundance was higher than 50% until 35 cm deep (Fig. 8).

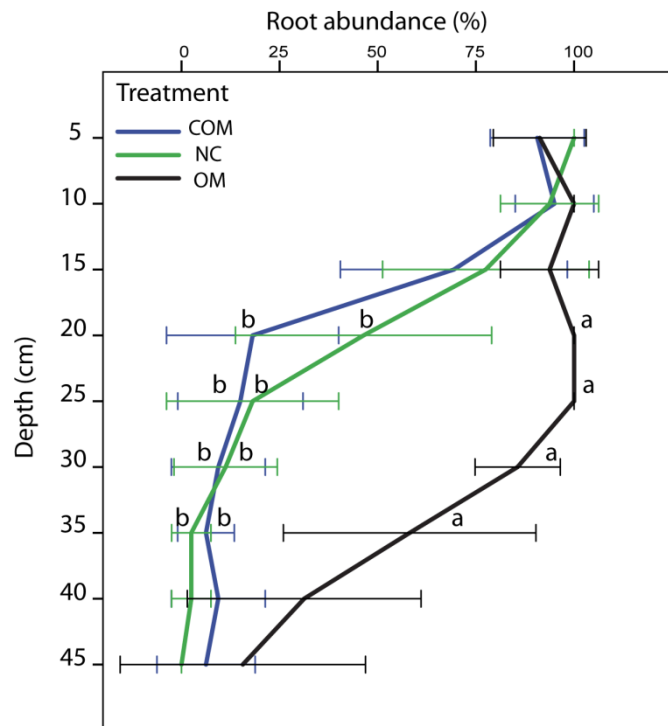


Figure 8: Vertical distribution of root abundance in the studied mesocosms. Different colours correspond to different treatments (blue, COM; green, NC; black, OM). Different lower-case letters mark significant differences between treatments for each depth.

PCA analysis on concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, N, Ni and P in different fractions of plant tissues (either roots or shoots) revealed three PCs that explained more than 81% of total variance in plant tissue composition. The concentrations of Fe, Mn, Cu, Co, Cr and Ni had positive contribution to PC1 (49% of total variance); whereas N, K and Mg had positive contributions for PC2 (21% of total variance). P had an intermediate contribution to both PCs (Fig. 9A). Ca had only influence on PC3 (11% of variance). The projection of plant samples on first two PCs showed a clear separation between plant fractions (roots and shoots) along PC1 (Fig. 9B). This suggests that higher concentrations of trace elements are present preferentially in roots. Two-way ANOVAs (factors treatment and fraction) were performed on elements related to PCs (Ni, Cr, Cu and P to PC1; K, Mg, N and P to PC2, Ca to PC3) (Fig. 10). No interactions were observed between the treatment and fraction (i.e. concentrations of each element had the same pattern of variation across treatments in roots and in shoots). Irrespective of treatments, P and K concentrations were higher in the shoots

than in the roots, whereas Cu, Cr and Ni were higher in the roots than in the shoots. Ca, Mg and N concentrations were similar in the roots and in the shoots. The plants from OM treatment had lower concentrations of Ca, Cr and Ni than other treatments, whereas K concentration in the roots and in the shoots was the highest (on average 8270 $\mu\text{g g}^{-1}$) in the plants from the OM treatment (Fig. 10).

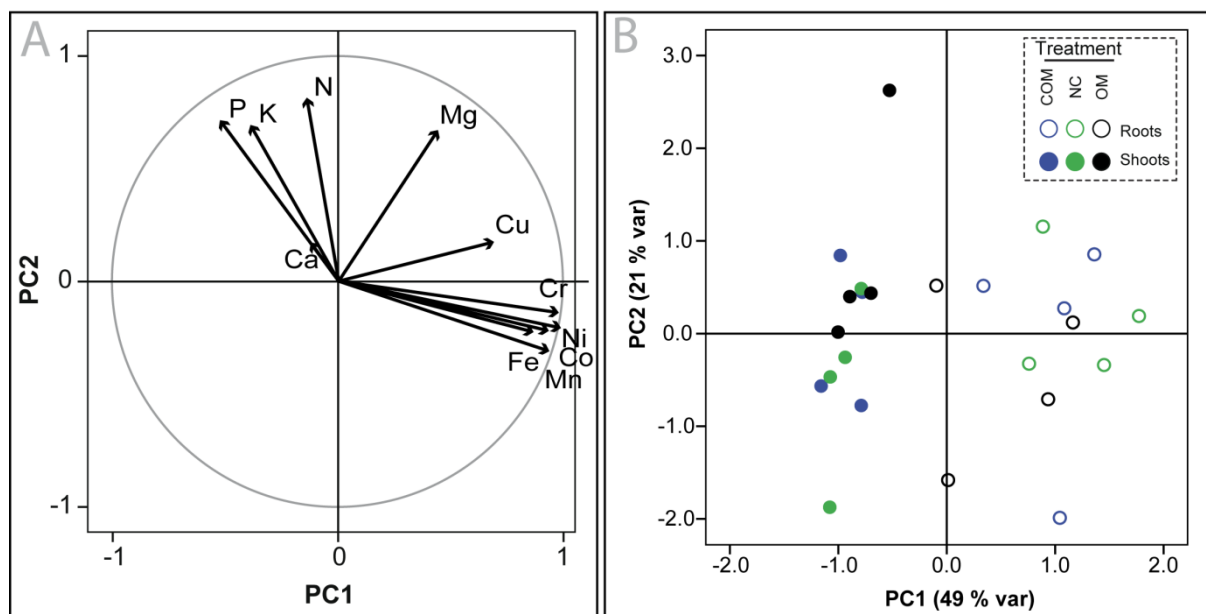


Figure 9: PCA of plant samples. **(A)** Loadings of each variable in two first Principal Components (PCs). **(B)** Projection of plant samples (each dot indicates one sample) on first two PCs. Different colours correspond to each treatment (blue, COM; green, NC; black, OM), whereas filled symbols indicate shoot samples and empty symbols indicate roots. Concentrations of different elements are abbreviated as their chemical symbols in (A).

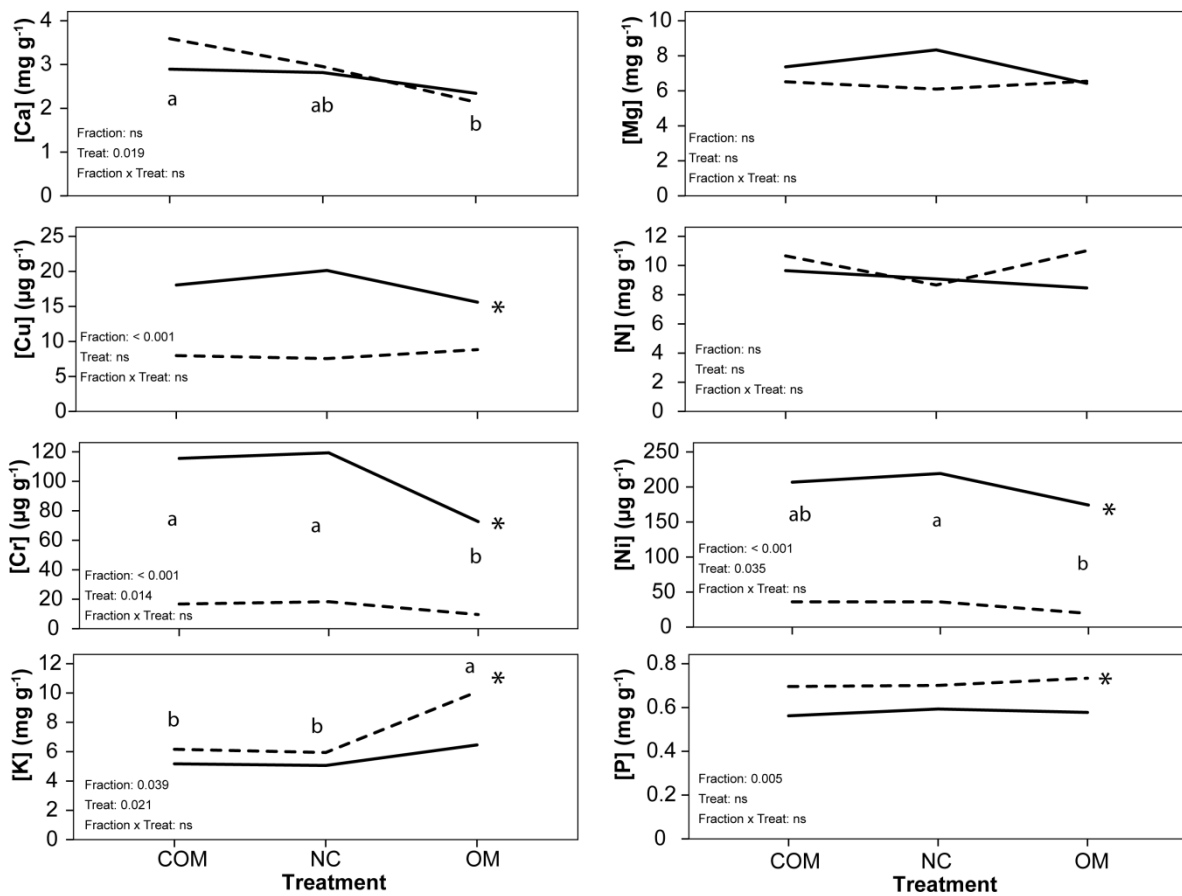


Figure 10. Variation in plant chemical composition. Each graph presents average concentration for each element in roots (continuous line) and shoots (dashed line) across three tested treatments. ANOVA results are presented in each graph as the p-values of the effects of the factors *treatment*, *fraction* (roots or shoots) and interaction *treatment* × *fraction*. ns: non-significant (p-value > 0.05). Asterisks mark significant differences among *fractions* irrespective of *treatments*. Different lower-case letters mark differences between *treatments*, irrespective of plant *fractions*.

Functions	Relevant indicators	Indicator values					Average rank			
		TOP	NC	OM	CON	UND	TOP	NC	OM	CON
Biomass production	Aerial dry biomass (g)	13.9 ± 3.0	19.4 ± 7.8	33.6 ± 14.6	0	nd				
	Plant cover (%)	77.9 ± 11.5	66.2 ± 23.3	91.4 ± 6.5	0	100	++	++	+++	0
Nutrient cycling	K total cumulative flux (g m ⁻²)	3.2 ± 0.7	3.6 ± 0.7	10.8 ± 2.1	nd	nd				
	K shoot concentration (mg g ⁻¹)	6.2 ± 0.8	5.9 ± 1.8	10.1 ± 3.6	0.0	6.7 ± 2.5				
	FDA hydrolysis at 20 cm depth (Abs490 units g ⁻¹ h ⁻¹)	0.9 ± 0.3	0.5 ± 0.6	23.7 ± 3.2	0.8 ± 0.7	77.1 ± 26.0				
	Alkaline Phosphatase activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	10.0 ± 7.5	20.1 ± 8.0	24.9 ± 6.8	13.1 ± 5.5	156.1 ± 52.9				
	Arylsulphatase activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	1.1 ± 0.6	2.4 ± 1.7	23.4 ± 4.8	1.8 ± 2.0	59.9 ± 21.7	++	++	+++	+
	β-glucosidase activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	18.6 ± 5.8	14.8 ± 7.6	14.5 ± 3.5	34.3 ± 19.3	62.2 ± 17.6				
	Urease activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	0.6 ± 0.4	0.4 ± 0.5	3.4 ± 2.0	2.0 ± 1.7	6.4 ± 3.3				
	Root abundance at 20 cm depth (%)	18.1 ± 21.9	46.4 ± 32.6	100 ± 0.0	0	nd				
Water filtration	Ni cumulative flux (mg m ⁻²)	11.3 ± 3.5	12.5 ± 1.2	56.8 ± 11.0	58.0	nd				
	Ni maximum leachate concentration (µg L ⁻¹)	34	20	81	250	nd				
	Zn maximum leachate concentration (µg L ⁻¹)	7	126	6	nd	nd				
	Exceedance of Ni critical limits (number of times)	1	1	36	13	nd	+++	+++	++	+
	Exceedance of Cr critical limits (number of times)	0	0	0	13	nd				
	Exceedance of Zn critical limits (number of times)	0	0	0	nd	nd				
Plant biodiversity habitat	Number of plant species	4.5 ± 1.3	4.8 ± 1.0	7.2 ± 4.6	0	6 ± 1.6				
	Presence of exotic species (% of total shoot biomass)	66.6 ± 18.0	66.3 ± 25.8	70.1 ± 25.4	0	0	+	+	++	0

471 *nd*: not determined

Table 5: Data used for the multi-criteria assessment of reclamation treatment effects. First column indicates the four ecosystem functions important for the reclamation of ultramafic dumpsites. Second column presents selected indicators that discriminate among treatments (and the units within brackets), followed by three columns with the average values ($\pm$ S.D.) of each indicator averaged for each treatment (COM, topsoil application + compaction; NC, topsoil + non-compacted; OM, topsoil + non-compacted + organic amendment), a fourth column (CON, control) showing the values in non-reclaimed tropical ultramafic spoils (data recovery from Raous et al. 2011 and Auber, 2017 *unpublished*) and a fifth column (UND, undisturbed) showing values for some parameters in an undisturbed ultramafic soil (five 4-m² square plots from mature *Ceuthostoma terminale* forest inside Kinabalu Park; Quintela-Sabaris, 2018 *unpublished*). Last four columns indicate the average ranks for each treatment and for each ecosystem function.

4. Discussion

Heavily disturbed soils, such as those in the study site, have reduced potential to provide ecosystem services because of the loss of soil functions (Echevarria and Morel, 2015). The recovery of ecosystem functions of disturbed areas may vary from passive to active reclamation measures, depending on starting situation and expected outcomes (Bradshaw, 2000; Rey Benayas et al., 2008).

In this study, three treatments, representative of realistic practices were tested (topsoil + compaction – COM –, topsoil + non-compacted – NC –, and topsoil + non-compacted + organic amendment – OM –) for the reclamation of a tropical Spolic Technosol on serpentinite. These three treatments can be ordered in a gradient of increasing reclamation effort, which implies inevitably increasing economic cost.

4.1 Biomass production

The establishment and increase of plant cover assist in the stabilisation of dump site soils, reducing the effect of wind and rain erosion (Tordoff et al., 2000), thereby improving ecosystem functions. After 12 months of the experiment, the plant cover was similar in the three treatments (average cover 78.5%), but it almost doubled observed plant cover in eight non-reclaimed ultramafic dumpsites and quarries in Sabah (48.3%, Quintela-Sabaris et al., *unpublished*).

505 The lack of differences in plant cover between treatments can be explained by the
506 beneficial effects of topsoil, which was applied to the three treatments. Topsoil
507 application is known to improve plant colonisation and growth in post-operation
508 environments (*e.g.* Echevarria and Morel, 2015; Losfeld et al., 2015; O'Dell and
509 Claassen, 2009). Indeed, 30 cm of topsoil is recommended to provide plants with
510 sufficient rooting depth (O'Dell and Claassen, 2009). In this study, we expected
511 that the main effects of the thin topsoil layer (just 5 cm) was acting as seed bank,
512 source of microbiota, and seedbed. However, topsoil application also improved
513 soil chemical and biological fertility parameters in the top 10 cm of soil profile, in
514 comparison with the deeper soil layers. Root development in the COM and NC
515 treatments was restricted to this nutrient rich layer, but in the OM treatment the
516 roots grew well (root abundance >50%) in the layer where oil palm fronds have
517 been applied. Despite of these differences in the vertical distribution, the root
518 biomass was similar between the treatments, whereas an increase in shoot biomass
519 (1.5 to 2 times higher than in the other two treatments) was observed in the OM
520 treatment.

521 The model of 'functional equilibrium' (Brouwer, 1962) states that plants allocate
522 more biomass towards shoots or roots to capture more of those resources that most
523 strongly limit plant growth. According to this model, it has been shown that plants
524 respond to a decrease in below-ground resources with increased allocation to roots
525 (*i.e.* decreased shoot:root biomass ratio) (Poorter and Nagel, 2000). Shoot:root
526 biomass ratio was higher in the OM treatment than in the other two treatments.
527 Thus, we can interpret that plants in COM and NC treatments, growing on nutrient
528 poor soils, allocated more biomass to roots to capture resources to grow; whereas
529 plants in OM treatment, as a consequence of the organic amendment, were less
530 limited by nutrients and could allocate resources to shoot biomass during growth.
531 In summary, topsoil application has fulfilled a major role in the restoration of plant
532 cover and biomass production, and organic amendment has provided additional
533 nutrients that reduced the nutrient limitations in the serpentinite soil.

534

535 4.2 Nutrient cycling

536 Towards the top and bottom of the soil profile, variables indicative of nutrient
537 cycling were similar between the treatments. At 10–25 cm depth, the main
538 differences were between the OM treatment and the other two treatments. Organic
539 amendment with oil palm fronds increased the soil total concentrations of N, Ca
540 and K (P and S were below the limit of quantification in all of the treatments).
541 However, the cycling of K and S appears to be most affected by organic
542 amendment: the cumulative flux and the concentration of K in roots and the root
543 and shoot S concentrations were higher in OM treatment than in the other two
544 treatments; whereas C and Ca concentrations were higher in COM and NC
545 treatments. Rooting depth increased greatly in OM treatment. This makes
546 vegetation less affected by drought and allows plants to explore more soil volume
547 for nutrient scavenging. Nevertheless, the most remarkable difference between
548 OM treatment and other two treatments in nutrients cycles was visible in the
549 improvement of bacterial activities in soil after application of organic
550 amendments: FDA hydrolysis (indicative of soil hydrolytic capacity) and enzyme
551 activities related to the cycling of important nutrients: nitrogen (urease),
552 phosphorus (alkaline phosphatase) and sulphur (arylsulphatase) were higher in OM
553 treatment. This effect has been observed in other metal-rich organic-amended soils
554 (e.g. Touceda-González et al., 2017) or degraded agricultural soils (e.g. Ros et al.,
555 2003) and can be attributable to the direct effect of the organic amendment or to
556 indirect effect *via* the increased presence of roots (and thus induced biological
557 activity due to root exudation) at that depth.

558

559 4.3 Water filtration

560 Ultramafic mine spoils may cause problems to groundwater pollution due to
561 leaching of Ni and Cr (Raous et al., 2010). Organic amendments have been widely
562 used on the reclamation of mine soils to reduce metal mobility and bioavailability
563 (e.g. Alvarenga et al., 2009a, 2009b; Burges et al., 2016). In this study, the OM
564 treatment reduced Cr soluble concentrations and cumulative flux, but induced an
565 increase of both parameters for Ni and the exceedance of the EU Ni-thresholds for

566 drinking water. Two processes may explain this unexpected effect of organic
567 amendment on Ni mobility: i) increased mobility of Ni due to slight pH reduction
568 (around 0.5 points); and, ii) the formation of soluble complexes of Ni with organic
569 compounds either released during degradation of labile fraction of the organic
570 amendment (Kala et al., 2009) or exuded by roots (Clemente et al., 2010). We
571 observed that drainage water samples from OM treatment had a dissolved organic
572 carbon concentration twice that of the other two treatments (data not shown). This
573 suggests that the second explanation is the most probable. It would be interesting
574 to develop a similar analysis using composted oil palm wastes (*i.e.* stabilised
575 organic matter) to determine which is the source of Ni-solubilising organic
576 compounds.

577

578 4.4 Plant biodiversity habitat

579 Spontaneous colonisation of post-mining sites has shown similar success as
580 technical reclamation (Bradshaw, 2000; Mudrák, O. et al., 2010; Šebelíková et al.,
581 2016). In some cases, plant communities recovered by spontaneous revegetation
582 were more diverse than reclaimed sites (Šebelíková et al., 2016). After one year of
583 the experiment, plant communities growing on the mesocosms had many
584 similarities with the site where topsoil was collected. However, three introduced
585 species (especially *Chromolaena odorata*) were dominant: on average 68% of
586 shoot biomass corresponded to introduced species across all treatments. Although
587 it is difficult to predict the evolution of plant communities from the results of one
588 year of colonisation, the results suggest that planting of native species that can
589 outcompete the invasive species is needed to meet a minimum objective for
590 creating a habitat for native plant biodiversity. The study of functional traits of
591 pioneer plant species on metal-rich disturbed habitats may help in the selection of
592 interesting species for reclamation (Ilunga wa Ilunga et al., 2015). Ongoing
593 research in tropical ultramafic areas on this topic points to perennial species with
594 shallow roots and lateral spreading capacity as the ideal candidates (Quintela-
595 Sabarís et al. unpublished). Grasses and sedges such as *Imperata cylindrica*,

596 sedges such as *Fimbristylis* sp. (Cyperaceae) or the fern *Pteridium esculentum*
597 appear to be good choices.

598

599 4.5 Global assessment of reclamation treatments on ecosystem functions

600 We tested three treatments, which represent increasing reclamation effort, and
601 their effects on four ecosystem functions were assessed, considering variables
602 from multiple compartments of the ecosystem. Our multi-criteria approach (see
603 table 5) indicates that the less intensive approach (compaction + topsoil) improved
604 plant biomass and water filtration functions with respect to non-reclaimed sites. As
605 noted above, the benefits of topsoil application have been demonstrated elsewhere
606 (Echevarria and Morel, 2015; Losfeld et al., 2015; O'Dell and Claassen, 2009).
607 Moreover, additional reduced compaction efforts did not lead to additional benefits
608 compared to topsoil application. In contrast, the most intensive treatment (non-
609 compacted + organic amendment + application of topsoil) had the greatest
610 improvements on nutrient cycling and biomass production functions, but at the
611 same time the performance of this treatment on water filtration was lower than the
612 less intensive reclamation approaches. Habitat for biodiversity function was
613 similar in the three treatments, but low due to the dominance of introduced species
614 in the mesocosms. Future reclamation actions should combine modification of soil
615 conditions and improvement of the starting plant community (*i.e.* seeding or
616 planting of native ultramafic species).

617

618 Conclusion

619 In this work, we have assessed the impact of three reclamation techniques for post-
620 operation ultramafic sites (*i.e.* topsoil application, non-compacted, and organic
621 amendment) on the level of four ecosystem functions. These four key ecosystem
622 functions for ultramafic areas were chosen according to our literature review:
623 biomass production, nutrient cycling, water filtration, and habitat for plant
624 biodiversity. A large set of measures on soil chemistry, microbial activity, plant
625 and water were measured to assess those ecosystem functions and an evaluation of

626 global ecosystem services would have mean a larger measurement effort (*e.g.* soil
627 physics, fauna). In order to process this large dataset of multiple variables we have
628 suggested a first approach for a multicriteria analysis. Such an approach would be
629 useful for the assessment of ecosystem services. However, the sensitivity of the
630 ranking and the computation have to be tested to assess the robustness of this
631 approach.

632 In the short-term of our study (one year), the three reclamation techniques all
633 improved the level of the ecosystem functions in comparison with literature data
634 on degraded sites.

635 The reclamation effort on a given site must balance the recovery targets, the
636 economic costs and the energy consumption. However, our results have shown that
637 increasing reclamation effort can be beneficial, detrimental or even have no effect,
638 depending on the considered parameter and ecosystem function. Longer studies
639 (5–10 years) are also needed to assess the evolution of the system in the mid and
640 long term. The recommendation of one reclamation approach over the other is not
641 straightforward, in the light of contrasting effects of our treatments. However,
642 generated information will be useful to selected choice the strategy for the
643 recovery of degraded tropical serpentinite quarries and dump sites.

644

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655

656

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Title: Effects of increasing reclamation effort on ecosystem functions of human-degraded soils from ultramafic areas

Journal: Journal of Geochemical Exploration

RESPONSES TO REVIEWERS' COMMENTS

Dear Prof. Ayuso,

Please find below the responses to the reviewers' comments on our manuscript. We have carefully considered and responded to each of the comments. Review comments are marked in *italics*, whereas our responses are in plain text.

Comments from the editors and reviewers:

-Reviewer 1

Review comments on GEXPLO_2018_215: Effects of increasing reclamation effort on ecosystem functions of human-degraded soils from ultramafic areas.

First, I should have refused to review the MS until it had line and page numbers. I had to add line and page numbers to allow line based review comments. Authors and Editors should be reminded that all manuscripts should have page numbers and line numbers before they may be accepted for review. In addition, there are numerous errors in English that should have been prevented by a colleague who actually knows English writing. Simple errors!

Sorry for the missing page/line numbers. When we read the Guide for authors of the journal, we didn't find any reference to page/line numbering, so we incorrectly assumed that numbering was not necessary. English usage in the reviewed version of the manuscript has been checked by an English-speaking colleague.

Page 4 is the page with normal text for a first page. I numbered each line with any text starting with 1 for the first line of the title.

The MS reports valuable research on an important topic, but needs considerable revisions.

The use of TOP for a "topsoil" treatment must be changed. All treatments were given 5 cm of ultramafic topsoil to provide a seed and microbe culture for the test. Perhaps COM for compacted would be appropriate, with UNC for "uncompacted" as the second treatment, and ORG for the third treatment.

Following reviewer recommendations, we have changed the codes for the treatments. New codes are: COM (for topsoil + compacted), NC (for topsoil + non compacted) and OM (for topsoil + non-compacted + organic amendment).

L13: Would it be clearer to say "Surface mining or other disturbing operations..."

Modification done.

L19: *“has been” should be “was”*

Modification done.

L24: *“treatment’s” should be simply “treatment”; use of possessive here is completely wrong.*

Modification done.

L26: *Delete “Our” and start with “Results....”*

Modification done.

L29: *Should use “gave” rather than “had”; and “improvements in” rather than “on...”*

Modification done.

L30: *Should be “of” rather than “on”*

Modification done.

L33: *Could add “historic” before “plant diversity”; and delete “feed”*

“Historic” not included. “Feed” deleted.

L34: *Should be “choice of the strategy....”*

Modification done.

L45: *Need space at end of sentence ...(2009).*

Modification done.

L48: *How about “post-disturbance” rather than “post-operation”? THROUGHOUT the MS.*

We prefer to use the term post-operation to refer specifically to human-drove disturbance on ultramafic areas. We think the term post-disturbance is more general, as it can also refer to natural disturbances (e.g. landslides), but in the context of the manuscript we wanted to talk specifically about disturbances caused by humans.

L52: *Why not list as: Ni, Cr and Co? Certainly Co is not the main problem with these wastes.*

Modification done.

L58: Need open parentheses before “Bradshaw....” Also suggest using colon after “necessary” and then the numbered list.

Modification done.

L60: How about “leaching” rather than “dispersion” of metals?

Modification not considered. We prefer the term “dispersion” as it refers to the spread of metals by wind erosion on bare soil or water erosion.

L63: Should start with “Diverse reclamation techniques have different....”

Modification done.

L64: Should be “effectiveness”; not “effectivity which is seldom used in English.

Modification done.

L74: “immigration” is not the right word here. Yes, seedbank and microbial inoculum are needed, but “immigration” does not describe this action of topsoil.

Modification done.

L78: Should be “also be”, not “be also”

Modification done.

L79: How about “remediation” rather than “construction”?

Modification not considered. We are referring here to the use of organic wastes and industrial byproducts for the construction of Technosols (see Séré et al., 2010). Moreover, remediation refers specifically to the removal of soil contamination which is not our purpose here (see e.g. Lima et al 2016).

L81: How about “reduction” rather than “alleviation”? And the pH of the ultramafic debris being studied here is actually slightly acidic, not hyperalkaline.

Modification done. In the text we used the term hyperalkaline referring to usual conditions that can be found on serpentine debris and hypermagnesian Leptosols from Borneo. Regarding the pH of the samples from our study, if we exclude the upper soil layer (where topsoil was applied) soil is neutral to basic (pH values between 7.3 and 7.9). And if we consider the pH of drainage water, values are basic to hyperalkaline (values between 7.5 and 9.2).

L100: “rainfall” is singular, not plural. Thus should be “2500 mm which is evenly distributed during the year.

Modification done.

L106: Collected “from” rather than “on”

Modification done.

L107: Should be “we collected ultramafic topsoil....” Obviously you “decided”

Modification done.

L112: Past tense, “were”, not “are”

Modification done.

L115: Consider “Organic waste was chosen....”

Modification done.

L116: Should be “second largest world producer....”

Modification done.

Figure 1 caption: Need a, b, or c labels which can then be referred to. The “Up” in the first line is not obvious from the Figure. Is that the Upper right sub-figure?

Labels added to the figure and legend modified.

L127: How about “sieved < 5 mm” rather than “at”

Modification done.

L128: Should delete the parentheses text and simply say “soil profile”

Modification done.

L129: Again, TOP is in incorrect label for this treatment compared to use of the same layer of ultramafic topsoil at the top 5 cm of all soil treatments.

Modification done.

L138: Should be “particle”, not “particles”

Modification done.

What was the size of the surface area of the two size “mesocosms” studied? Shape? And is the sand layer included in the listed height of the soil column in each test vessel? So the 0.054 m² is not enough to communicate what was used in terms of surface dimensions. Round? Square? 0.054 m² would be a 23.2 by 23.2 cm vessel, or nearly 10 inches if square. The large replicate was only 29.7 cm², so not all that different?

The mesocosms were constructed in cylindrical vessels. Three vessels per treatment had a diameter of 39 cm, whereas the fourth had a diameter of 50 cm. The reported volumes referred only to the soil (excluding sand layer). The information about the surface and shape of the mesocosms has been added in material and methods.

The bulk density of the organic matter amended soil would strongly affect the volume of this treatment, so the weight of soil and palm leaves added as the “OM” layer should be made clear. So the bulk density of each the UNC layer, the COM layer and the OM layer should be clear to readers, and the bulk density of the non-OM layer of treatment 3 should be clear as well.

We agree with the reviewer that bulk density would be interesting to assess more precisely some of the studied ecosystem functions. During experiment set up, we were very careful about keeping the same volumes for all replications. Unfortunately, no heavy duty scale was available at the remote Monggis substation, so we were not able to determine exactly the masses of each material. We included in the text a short sentence about the possible differences in bulk density between treatments. However, we consider that thanks to the high amount of parameters measured in drainage water, soils and plant samples, we have enough information to interpret the main effects of the reclamation treatments we applied.

In Table 1: No number should have more than 3 significant figures!!!!. (levels of C, C:N, S, Al, Fe and Mn have 4 significant figures). Also, % is not a metric unit so these should be in g/kg. The 0.04% P in the palm leaves is quite low for good plant nutrition of most species; usually 0.1% P (1 g/kg) is the minimum of healthy leaves, while 1.5 g/kg is reasonable fertility.

Significant figures and units have been corrected. P concentration in dry palm leaves is low, but this is not necessarily negative in our specific situation. Ultramafic soils have very low P concentrations *per se*, and ultramafic flora is specifically adapted to that condition. Excessive fertilization may have a negative effect by favoring the colonization of ubiquitous ruderal plants.

Also, palm leaves would likely contain lower than 100 mg Fe/kg, so these leaves were quite “dirty” with soil particles which could supply microbes to that treatment directly. What were the Cr and Co levels? What was the Ni level in the “palm leaves”?

We quantified a Fe concentration around 500 mg kg⁻¹, but this doesn't mean the leaves were dirty: Fe concentrations in oil palm samples reported by other authors were even higher: around 1000 mg

kg⁻¹ in leaves, trunk and empty fruit bunches (Kala et al. 2009) and 1% Fe in shredded empty fruit bunches (Baharuddin et al. 2009). In fact, we paid attention and collected only leaves without evident soil “contamination”. We have added to Table 1 the concentrations of Ni (29.1 mg kg⁻¹), Cr (9.07 mg kg⁻¹) and Co (2.78 mg kg⁻¹). Palm leaves were collected from a farm on ultramafic soil. If there was soil “contamination” on the leaves, we would expect high concentrations of Ni and Cr. However, these values are low indicating that leaves were quite “clean”. Regarding microbes, palm leaves were not sterilized before shredding and mixing with soil. Thus, bacteria and fungi were present in the leaves when we applied it, for sure! This is the same situation as with any other organic amendment applied to a soil, unless the amendment is sterilized before application. Anyway, we consider that the biota contribution by the palm leaves is low compared to the fresh topsoil layer we applied to each treatment.

Figure 2: This Figure is very helpful but the treatment labels need to reflect the actual treatments as noted above, the TOP has only 5 cm of “topsoil” and that treatment is equal for all 3 treatments.

Treatment labels have been modified.

L154: The soil samples should be described in terms of top and bottom levels, 0-10 cm, 10-20 cm and 20-40 cm if that is what is meant by the text shown.

Modification done. The levels have been corrected: 0-10 cm, 10-25 cm and 25-45 cm.

L155: Need to clarify that all root material from the whole vessel was collected as one sample of roots.

Modification done.

L161: Were the water samples acidified after filtration in the normal protocol to preserve metals for analysis?

We didn’t acidify the samples to be able to also measure the dissolved organic carbon in the drainage water. Due to long delay (more than a month) between sampling and analysis, the concentrations of dissolved organic carbon were not precise enough to be presented in the manuscript, and we used that information only in the discussion as an explanation for the increased Ni mobility in the OM treatment. In spite of no acidification, samples were transparent and no flocculation was observed prior chemical analyses. Thus, we think that our results are reliable although metal concentrations may be downbiased. A sentence has been added to material and methods to explain this point.

L169: The first sentence is a repeat from above. Delete.

Yes, this sentence appears as a repeat, but actually it isn’t. This is not a repeat. As indicated in section 2.3, when we constructed soil profile, topsoil and serpentine debris were sieved (respectively at < 5 mm and < 25 cm) and palm leaves were cut in pieces of around 2 cm diameter.

Therefore, the soil we collected from the vessels at the end of the experiment had different granulometries and we sieved (< 5 mm) to obtain homogeneous samples for analyses.

L170: The normal use of capitals should be followed here. Alkaline Phosphatase, etc., are not capitalized at this position in a sentence. And the rest of this paragraph has many Capitalization errors.

Errors corrected.

L178: Is there a reference for the “microplate” method to measure these activities?

Reference added (Dick et al. 2013)

L190: Is there a reference for this standard method?

Reference added (Aran et al. 2008)

L198: The term “completed up” may be a French term; the English term is “diluted to”. This error occurs in several places in the MS. Also L206.

Error corrected.

L206: Did you not measure Mn, Fe and Cu in these samples? These data would help readers.

Yes, those elements were measured. We have included info about these elements in the new version of the manuscript.

L212: Need a period after “size.”

Error corrected.

L215: Authors note use of ANOVA several times, but present mostly PCA results. Reviewer believes that an ANOVA would better communicate the significance of the treatment differences. A correlation matrix would also show the important relationships among variables to readers as clearly as the PCA plots.

In this experiment we have produced several multivariate datasets: composition of leachate water, soil, and plant samples. We have used multivariate PCA analysis to assess the complex information contained in those multivariate datasets and reduce it to a two dimensional space determined by two first Principal Components (PCs). In our opinion, PCA offers advantages over correlation matrices, since with PCA we can estimate at the same time the relationships between variables (inset graphs with the loadings of variables on each PC) and the relationships between the samples (graphs with samples plotted against first two PCs). In all cases, we have complemented PCAs with ANOVAs.

In the case of drainage water, we compared the concentrations of the main trace elements in ultramafic soils (Ni, Cr and Co) and results of ANOVAs were reported in the original version of the manuscript. In the other two cases (soil and plant parameters), we could have performed ANOVAs on the PCs, since they are compound variables that summarize most of the information in the data. However, we rejected this option because the results would be difficult to understand for the readers. Instead, we selected one variable with high contribution to each PC and then we performed ANOVAs to compare differences between treatments for that variable. We explained this approach in the results section where we presented soil data results. We have slightly modified the text to also make clearer this approach in plant dataset, a new figure with plant data and results of ANOVA analyses has been included.

L218: Need to use “on the soil dataset”

Error corrected.

L220: Here the “at 20 and 40 cm depth” is unclear. Reviewer suspects that the authors are referring to the 10-20 cm soil and 20-40 cm soil samples. Not the soil specifically at 20 and 40 cm depth.

Modification done. We meant that soil samples were collected around that depths, but the correct expression is that the soils were collected at 0-10 cm, 10-25 and 25-45 depths.

L225: Could use “vessel” or “replicate” rather than “pot” here. Did not call them “pot” above.

Modification done.

L232: Consider adding “and” after “diversity,” and before “root and shoot....”

Modification done.

L235: Again, ANOVA should be included.

ANOVA results has been included in Figures 4, 6, 7, 8 and 10 and in Table 4.

L236: If data for Cu, Fe and Mn are available, they should be included here and in the data reported.

The requested data have been included and PCA analyses of plant chemical composition has been re-run.

L239: Again it should be “treatment” effects, not “treatment’s” effects. Not a possessive case.

Error corrected.

244: *Should be: Trace element and nutrient concentrations in soils were chosen as explanatory variables to aid in the interpretation of treatment results.*

Sentence corrected.

In Table 2 (did not number these lines): the root abundance at 20 cm seems less useful than rooting depth mean for the 4 reps.

We consider that root abundance at 20 cm is an interesting parameter. We observed that roots develop usually on the top 10 cm of soil. The presence of deeper roots (and their abundance) has implications in water infiltration, in tolerance to drought and in stimulation of biological activity in the soil. Use of average rooting depth (calculated on the basis of the deepest root found in each vessel) has its limitations: *e.g.* the presence of one deep root in one of the vessels in one of the treatments would bias the result. Finally, we want to clarify that root abundance at 20 cm was estimated as an average for the 4 reps in each treatment.

And the maximum concentrations of Ni, Cr, etc., is not the most relevant outcome. Over time. Leaching gives an average composition. And the Raous (2010) paper showed that upon disturbance the ultramafic mine debris give much higher metal levels in leachate until the soil chemistry settles after disturbance. Did the average leachate composition exceed standards?

In Table 2 we included several indicators for each ecosystem function. Besides maximal concentration of Ni, Cr etc. we also included the total cumulative fluxes of those metals. This second parameter accounts for the presence of trace elements in leachate water over time. Regarding the results of Raous et al (2010) and the exceedance of standards, please refer to authors' response to comments on EU limits for drinking water.

L249: The volume of water per mesocosm should be the cm of water because mesocosms varied in surface area so the volume would be appreciably different among reps. Could express as L/m² of surface area for each vessel?

Modification done.

L251: “analysis of”, not “analysis on”

Corrected.

L252: How about “variance of drainage water composition.”

Modification done.

L254: Consider “reflects” in place of “had only influence on” PC3....

Modification done.

Figure 3 and others: The use of these grey lines is confusing. One can use continuous, dotted and dashed lines in a dark color. Color lines would be helpful and the variance bars in Fig B need to be described? Standard Deviations? Errors? All lines in the Figures need to be darker so that they are readable in the smaller versions in a publication.

Color lines have been included in this figure and others. In order to make the figures clearer, we have eliminated gray lines corresponding to volume of drainage water. Information has been included in the figure legends to indicate that variance bars represent standard deviations.

L272: Report the detection limit so readers know what you really measured. Also, a semicolon should follow the “Cr and Ni;”

Modification done. We report quantification limit.

L278: “overpassed” is not English. Should be “exceeded the EU....”

Error corrected.

L279: Reviewer checked the EU limit cited and found 70 µg/L as the EU limit. And the US-EPA limit for Ni in primary drinking water promulgated in 1995 was 0.5 mg/L for long term protection of children but the MCL was 0.1 mg/L (EPA 81.1-F-95-002m-T, October 1995). The limit reported in the MS is 0.02 mg/L which was exceeded by some samples. But as noted above, the average Ni level in the leachate water was not likely exceeding the 0.02 figure noted. And the Raous paper noted above showed remarkable drop in leachate Ni from Brazilian mine materials with much higher levels of Ni than in the soil materials in the present MS.

As a minimum, the citation for any valid drinking water Ni limit should be provided and verified to give the official document which lists the number.

After reading this comment, the authors have re-checked the EU directives and have confirmed that the information provided in the original submitted manuscript is correct, and correctly referenced to a European Directive that is currently in force: the chemical standard values for drinking water in the EU, according to the Council Directive 98/83/EC (annex I, part B) are 20 µg L⁻¹ for Ni and 50 µg L⁻¹ for Cr. This directive was published in the Official Journal of the European Communities on 5.12.98. This directive is named and referenced in the text.

Regarding Ni concentrations in leachate water, if you have a look at figure 4A, you will observe that in 10 out of 12 months, average Ni concentration in leachate water from treatment OM was higher than the EU threshold for drinking water (20 µg/L)

Our data are in clear accordance with those on Raous et al (2010). Raous et al. (2010) reported the results of two column experiments. Columns were 30 cm depth and 4.2 cm internal diameter. In the first one, with columns filled with non-fertilised mine spoils, they saw a drop in Ni concentration (from 3 mg/L to less than 0.5 mg/L) during the first month of experiment and then Ni values stayed

below 0.5 mg/L until the end of experiment. Raous et al explained this effect as a result of the dessication/humification of mineral samples (starting material was dried and sieved at 6 mm before the experiment setup). In our experiment, we didn't dry the serpentine debris and we sieved it to remove rocks bigger than 25 cm diameter. Ni concentration in leachate water from treatments COM and NC (without organic amendment) were low (around 0.01 mg/L) without any important variation along the duration of our mesocosm experiment.

Raous et al (2010) also reported a second experiment where mineral fertilisation (ammonium nitrate) was applied to a column with a mixture of Ni-laterite mine spoils. They observed that 30 days after the application of fertiliser, Ni concentration in leachate rose up from 0.1 to 0.6 mg/L. They interpreted this as a result of a ionic balance (increase of NO_3^- concentration induced a mobilisation of cations -including Ni- to keep electroneutrality of soil solution). In our experiment, application of organic amendment increased the concentration of Ni in leachate water. We discuss in section 4.3 that this fact can be caused by the formation of soluble complexes of Ni with organic acids released during degradation of oil palm leaves, or exuded by roots.

L283: It is not clear why the flux is defined in terms of mg/m²? Drinking water limits are in mg/L. Chromium leaching is either Cr⁶⁺ which can be soluble or adsorbed on fine particles, while Cr³⁺ is a constituent in fine particles which should not have reached the filtered water samples?

Both types of information were given in the original manuscript: concentrations and cumulative fluxes. Concentrations were related to the drinking water limits, as indicated above. Concentrations were expressed in mg/L. Fluxes were used to express the actual amount of Cr or Ni that were leached in drainage water, to eliminate the effect of monthly variation in volumes of drainage water as well as the difference in surface for the mesocosms. Different barriers were applied to prevent the presence of soil particles in the drainage water (two geotextile layers in the vessels, plus filtration of collected samples). Thus, detected Chromium was soluble in the drainage water. Chromium concentration was very low in all the drainage water samples.

L283: the flux “rose”, not “raised”

Error corrected.

Figure 4: The comments on Figure 3 apply here as well. Should use dark lines with different patterns; In the bottom Figure of both A and B, there are 4 lines but the text notes only 3????

Figure 4 has been corrected and colours added.

L206: Again, ANOVA would be clearer to most readers and provide the critical information needed to understand the findings. The line says that three PCs explained 92% of the variance. BUT VARIANCE OF WHAT does this refer to? Of each metal? Or of all metals? Why are not P, Mg and Mn included in this analysis?

We provided an explanation on our choice of statistic analyses above as well as in the text of the manuscripts (lines 244-247). Mg and Mn were included in the PCA analyses. PCs explained 92% of

variance of multivariate soil dataset (which included chemical and biological parameters). P was excluded of the PCA because, as we explained in the manuscript (section 2.6 Data analysis), P concentrations in samples at 10-20 and 20-40 cm depth were below quantification limit (approx. 100 mg kg⁻¹). The whole soil dataset included 26 variables. Instead of performing 26 two-way ANOVAs, we performed a PCA to summarize all this variability and then, as we indicated in the manuscript (first paragraph after Figure 5, lines 361-363) we selected three soil variables with high contributions to PCs (FDA degradation, positive contribution to PC1; pH, negative contribution to PC1; exchangeable K, contribution to PC2) and performed the ANOVAs on them. The other 23 variables were highly correlated to one of the three variables we selected, so it is expected that they will show same differences between groups and treatments as the three variables which we performed the ANOVAs with.

L301: This text indicates that the two layers below the added topsoil in the ORG treatment were sampled separately and analyzed separately. This is not what was described in the Methods and Materials. Were both of these layers in the OM treatment UNCOMPACTED or DECOMPACTED? Needs to be made clearer in the methods section and in discussion. The text says “middle”, but a more definitive description should be used. 5 cm of “topsoil” on top; 5 cm of sand on the bottom; and two sections of debris plus OM (of what density) and of debris without OM were in the Middle.

In the material and methods (section 2.4) we reported that three soil samples were collected at three depths (10, 20 and 40 cm) from each mesocosm. As we said above, we meant that the soil samples were collected within this depth intervals 0-10, 10-25, 25-45 cm. We performed the same sampling for the three treatments, to compare differences in the profile caused by uncompaction or by organic amendments. We understand that the use of “middle” may cause confusion in the readers, so we have corrected it to express correctly the depth (10-25 cm)

Figure 5: It is the PCA of “soil measurements”, not “soil samples”. Again, the use of gray causes confusion. Color would be much better to communicate the treatment effects. And lines need to be thicker, etc.

Issues corrected.

Figure 6: This is also confusing in white, gray and black. Color would work better. The depth of the samples are hard to understand in this Figure. If the top 10 cm were sampled together, that would be the added topsoil plus 5 cm of the uncompacted or compacted soil, or of the OM amended soil. That is the top 10 cm. The next section, 10-30 cm apparently, would be mixed in the ORG treatment, while the bottom from 30-50 cm should be less confusing? Somehow this description needs to be corrected, or the unexpected mixing of materials in the sampled layers needs to be justified. And the significance test for differences across all depths needs to be clearer to readers.

Issues corrected in the figure. Sampling depths were 0-10 cm, 10-25 cm and 25-45 cm, measured from the soil surface (topsoil layer is included in the measurements). Although only 5 cm of topsoil were added, biological activity (mainly root development) along the duration of the experiment made that, at harvest, we observed in all treatments a surface layer (from 0 to around 10 cm depth) with a very different colour (reddish/brown, contrasting to the greyish-blue of the serpentine

debris). This information is now included in the manuscript. We collected this layer separately. Then the other two layers 10-25 and 20-45 corresponded to the layer with organic amendment, and to the bottom of the profile with only serpentine debris. Sand layer was, of course, excluded from any analysis. We have modified the text to avoid possible misinterpretations.

Plant diversity: The authors applied the same well mixed “topsoil” as a 5 cm deep cap on the “soil” columns in this test. As such, that “topsoil” should have been a relatively uniform source of seeds for plant species and soil microbes and fauna. Thus the expectation would be that the same plant species would try to grow on all of the treatments. Later when growth depended on nutrients and water supplied by the “test soil columns”, treatments could have an effect on survival of the plants which established from the seed stock of the “topsoil”.

The reviewer expressed the normal expectations in plant diversity due to application of topsoil. However there are other factors that may have affected the results. On one side, the vessels were placed under open air conditions, so new seeds may have arrived from plants growing on the vicinity of the mesocosms. On the other side, germination was delayed in the OM treatment (maybe due to an allelopathic effect of palm leaves) and this delay may have affected the composition of plant community (i.e. species with short-living seeds may have reduced its presence in soils with palm leaves).

Figure 7: This figure is very confusing. “Colours refer to the online version of the Figure.”, but the colors and PCA definitions are at best confused.

This figure has been displaced to the Supplementary material section. Figure has been modified to make it clearer, and legend has been modified.

L318: “Shoot” should not be capitalized.

Corrected.

L319: The “(amendment with organic matter)” has been defined clearly above and is not needed here. And it was only a layer of the test soils that was treated with raw leaf OM with very high C:N ratio which would release little N for the crops. Perhaps the P, K, Ca and other nutrients from the Leaves caused the observed effects?

Corrected.

L320: No more than 3 significant figures: 33.58!!!

Corrected.

L324: “in each treatment” would mean that among reps, the plant species varied “in each treatment”. Likely that the authors mean “among treatments”

Corrected.

L326: Look up “apparition” in English dictionary; should be “appearance”

One of the meanings of apparition in the dictionary is “the act of becoming visible”. Anyway, we change the word.

L330: With one larger mesocosm per treatment, were the number of individuals at day 300 less than 25 for each experimental unit? Should also be “fewer than” rather than “less than”

The information presented is correct. Several big plants occupied a big percentage of the surface of the mesocosm, and some germination flushes provoked a high increase in the number of plants. However, during short-dry periods there was high plant mortality, especially among the seedlings. Text corrected.

L332: How about “but during the last month the number....

Text corrected

Figure 8: Again the use of solid, dashed and dotted lines rather than gray would be much clearer to readers. And other admonitions about the Figures above.

This figure has been corrected to make it clearer.

L350: The information here is not clear. Were roots present at 35 cm below the surface in the OM pots, or only is 50% of the OM pots? UNCLEAR.

As we indicated in the material and methods, root depth was assessed using a transparent plastic layer with a 5 x 5 cm grid. 50% root abundance at 35 cm means that on average (over the 4 reps), at that depth, roots were present in 50% of the 5 x 5 cm squares.

Figure 9: What are the units for “Root Abundance”? Is this mass or length or surface area of the roots? How get 125%? And use of clearer colors or marks for lines would help.

Units (%) added. Root abundance (at each depth level) was calculated as a ratio of the number of squares where roots were present over the total number of squares per depth level. Colours included to make the figure clearer. No value was higher than 100%, in some cases, error bars (representing SD) exceed the 100%.

L347: The higher levels of trace elements in roots is simply the unremovable soil particles which contaminate soil grown roots.

As indicated in Material and methods, roots were thoroughly washed with tap water and rinsed with deionised water, so we expect that the effect of “unremovable soil particles” is low and it would have affected our results in the same way as any other published paper that analysed root samples. Exclusion (*i.e.* preferent accumulation of trace elements in roots) is a common strategy of metal tolerance in plants. Indeed, most of the species on ultramafic areas are excluders.

Figure 10A: Is this the total biomass element concentration? Of the shoots or roots or average of shoots and roots? The localization of elements is hardly unexpected. Those in the upper left and near are all readily translocated into shoots, while those of the right are elements hardly translocated to shoots of plants.

For PCA we used a dataset that included the chemical analyses of root and shoot samples separately. As we said above, localization of elements (*i.e.* trace elements preferentially in the roots) is common in metal excluders. If any Ni-hyperaccumulator had grown in the mesocosms, the pattern would have been different.

L357: What are the “expected outcomes” here? Does this mean the desired outcome of a persistent vegetative cover similar to the local ecosystem?

In the context of the first paragraph of the discussion “expected outcomes” referred to the different uses the land manager want to develop in a reclaimed serpentinite soil, from the recovery of a vegetative cover similar to local ecosystem to the cultivation of hyperaccumulators to develop agromining or the cultivation of high biomass crops for energy production.

L372: “testing” rather than “experiment”?

Corrected.

L374: This is confusing. If the root biomass was similar or “not different” among the 3 treatments, but the roots went much deeper in the ORG treatment, was the root biomass similar in treatments?

Root distribution along soil profile was different between treatments. In treatments COM and NC, dense root development occurred at the top 15 cm of soil profile only. In treatment OM roots developed well in the layer where palm leaves were applied and also below, but the roots were less dense than in other two treatments. For our comments on root biomass, please see our response to your comment on L380.

L378: How about “dumpsite soils”

Corrected.

L380: root biomass vs. root depth? Did the deeper roots provide higher shoot biomass and nutrient levels in the OM treatment? If so, is the text technically correct.

Upon this comment from the reviewer, we noted that this point was not clearly explained. We have enlarged this section of the discussion to better explain this point. We have completed table 4 with the assessment of differences in shoot:root ratio between treatments. Plant biomass allocation to root or shoot growth is affected by environment. The model of ‘functional equilibrium’ (Brouwer, 1962) states that plants allocate more biomass towards shoots or roots to capture more of those resources that most strongly limit plant growth. According to this model, it has been shown that plants respond to a decrease in below-ground resources with increased allocation to roots (*i.e.* decreased shoot:root biomass ratio) (Poorter and Nagel, 2000). Shoot:root biomass ratio was higher in the OM treatment than in the other two treatments. Thus, we can interpret that plants in COM and NC treatments, growing on nutrient poor soils, allocated more biomass to roots to capture resources to grow; whereas plants in OM treatment, as a consequence of the organic amendment, were less limited by nutrients and could allocate resources to shoot biomass during growth. In summary, topsoil application has fulfilled a major role in the restoration of plant cover and biomass production, and organic amendment has provided additional nutrients that reduced the nutrient limitations in the serpentinite soil.

L387: Top 10 cm was a mixture of “topsoil” and uncompacted, compacted, or uncompacted with added OM. Should make this clear in the descriptions of the outcomes.

Comment added in the new version of the manuscript.

L388: “in the first top 15 cm”; at least delete the “first” – first and top have same meaning here. If the root growth was similar in the top 15 cm of all 3 treatments, but roots grew deeply into the OM treated soil layer, how can the roots be equal among treatments?

L389: Roots did not so much develop preferentially in the top 15 cm, as stopped growing into the severely infertile subsoils of those treatments, but grew well into the OM treated layer. Roots were “limited to shallow soil in the two treatments, but not the third.

Paragraph corrected.

L397: Authors state that P and S were below quantification levels in all treatments including the OM treatment. But P detection limits are below 1 mg/kg, and the leaves contained 400 mg P/kg. If total was measured, how was P not measured? Surely the OM treatment added much more exchangeable Ca to the soil layer with OM than was present in the other treatments?

Limit of quantification of P was 105 mg kg⁻¹. Although an increase in P was detected in soil samples where palm leaves were applied, P concentration was below quantification limit (the estimated value was approximately 50 mg kg⁻¹). Regarding Ca, we indicated in the first version of the manuscript that palm leaves application increased total soil concentrations of N, Ca and K.

L403: “anyway” seems inappropriate here.

Corrected.

L417: Biological activity and this pH would favor rapid reduction of chromate to chromic so the effect of OM on Cr leaching is hardly unexpected.

Yes, but we made reference to Cr to contrast the reduced mobility of this element to the increased mobility of Ni.

L430: Spontaneous vegetation is either from the seedbank or from wind borne seeds. Were there any limits on wind blown seeds reaching the test soils?

Vessels were placed on open air conditions. We didn't placed any barrier to limit the arrival of wind borne seeds to mimic real soil rehabilitation technics.

L483: Need "the" before "experiment"

Corrected.

L443: The role of shallow spreading roots vs. deeper rooting plant species is controlled by the limited nutrient supply of these disturbed sub-soil materials. Roots can grow with supply of Ca and some other nutrients only so far before the growing plant cannot support further seeking of needed nutrients. If sub-surface soil materials such as the ORG treatment of this experiment were applied, roots were proliferate deeper than usually seen in the disturbed mine waste deposits.

Please see above our response on root biomass vs. root depth for an answer to this comment.

L452: Authors say that "application of topsoil improved plant biomass.... But they did not test "application of topsoil". 5 cm of topsoil was applied on all treatment mesocosms. This statement is unjustified.

The sentence has been modified to avoid confusion.

L453: "As we already mentioned" should be "as noted above...."

Corrected.

L454: "exposed" should be "revealed".

Corrected.

L457: "greatest", not "best" improvements. Best is a human judgement.

Corrected.

L466: *Again, the treatments did not test application of topsoil as topsoil was applied to all treatments!!!!*

In our experiment, we tested three treatments with increasing reclamation effort (topsoil + compaction; topsoil + non-compacted; topsoil + non-compacted + organic amendment). Our intention was to underline that just topsoil application (with no other action) had positive effects, whereas topsoil + uncompaction yielded no additional benefits. Sentence has been modified to avoid confusion.

L470: *“has been needed” should be “were measured”.*

Modification done.

L476: *“seems” should be “seem”*

Corrected.

L481: *“...possible affection to humans”? Desired by humans? Demanded by legislation?*

Sentence has been corrected.

L484: *“economic dispenses” does not communicate in English.*

Error corrected.

L485: *“other”, not “others”*

Error corrected.

Some references are incomplete despite being published well before 2017. The van der Ent 2015 needs volume and pages.

Errors corrected.

Table S1: The statement “X at 20 cm” is not a valid statement as the soil which was assayed for various measurements was from either the 0-10 cm, the 10-30 cm or 30-50 cm depth? Throughout the Table.

Table modified.

-Reviewer 2

The study data is worthy of publication, but structure and composition of the manuscript is disorganized. I found it very difficult to understand the study as the manuscript was written. Clarification and more concise wording is needed. The manuscript should be reviewed by someone fluent with English composition prior to resubmission.

English usage has been deeply reviewed and improved. We developed a complex study with several measured parameters (water, soil, plant chemical composition, plant diversity, soil biological activity) so it is also complex to present all the results in a simple way (e.g. several subsections in the results and discussion were needed). Effort has been made in improving the structure.

-Reviewer 3

The present work of research has as a focus to compare three different methodological proposals of recovery ultramafic areas strongly impacted by anthropic activity. The three proposals simulated different efforts to recover the degraded environment. With a holistic approach, the authors proposed access to key ecosystem functions from ultramafic areas, through chemical, physical and microbiological soil determinations, biomass production and water flow. The results obtained were subjected to multicriteria analyzes that allowed to compare the different systems proposed, in a really interesting and innovative way.

However, as proposed by the authors to analyze and integrate analytical results for the various functions accessible ecosystem (nutrient cycling, biomass, etc.), I felt it difficult to verify the order of magnitude of some of the different variables used in the statistical analysis. In this sense, I consider it important to include in the body of the text, Table SI, as a Table 5. Also, I recommend that this table contains descriptive data for each variable, maximum and minimum values (or average $\pm$ SD), relative to each treatment. And, if possible, that values of the same parameters related to the intact (without disturbance) ultramafic area of similar climatic regions, obtained in the literature, should be included. This would facilitate the perception of the degree of initial degradation and the changes that have occurred over the time of the implementation of recovery systems.

Finally, regardless of the efforts made in the multi-criteria analysis used to compare the treatments (recovery techniques), I agree that any conclusion regarding the functioning of the systems is very fragile and premature due to the short time of implementation in nature (12 months).

Table S1 has been moved to the main text (now is table 5), and SD values have been added. As the reviewer underlines, we have indicated in some places of the discussion and conclusion that a longer time frame would be needed to estimate the evolution of the reclaimed system and its functioning.

-Reviewer 4

Dear Authors

The manuscript is a soundly designed experiment, which ultimately shows that reclamation by topsoil can be improved through the addition of organic material. This is not a novel finding, but by considering the positive and negative effects of such amendments within biogeochemical cycling makes this manuscript of interest. It is refreshing to see, for once, that in-depth research was conducted to really pull apart the movement of metals within the system. Hence, the caution applied

throughout the manuscript is excellent in highlighting that when amendments are sought, that the purpose of the reclamation and the recovery strategy should be considered in detail. Not all amendments are beneficial under all conditions is a very good finding.

Throughout the discussion and conclusion I did feel that it was not always to the point and drifted from the findings of the manuscript. Your conclusion should indicate that the major outcome was that increasing reclamation effort can be beneficial, detrimental or even have no effect, depending on what you measure. As a recommendation from your findings you should then suggest that purpose and strategy should consider this. Currently as it stands the message is not conveyed fully.

Please see the attached manuscript with suggestions and corrections.

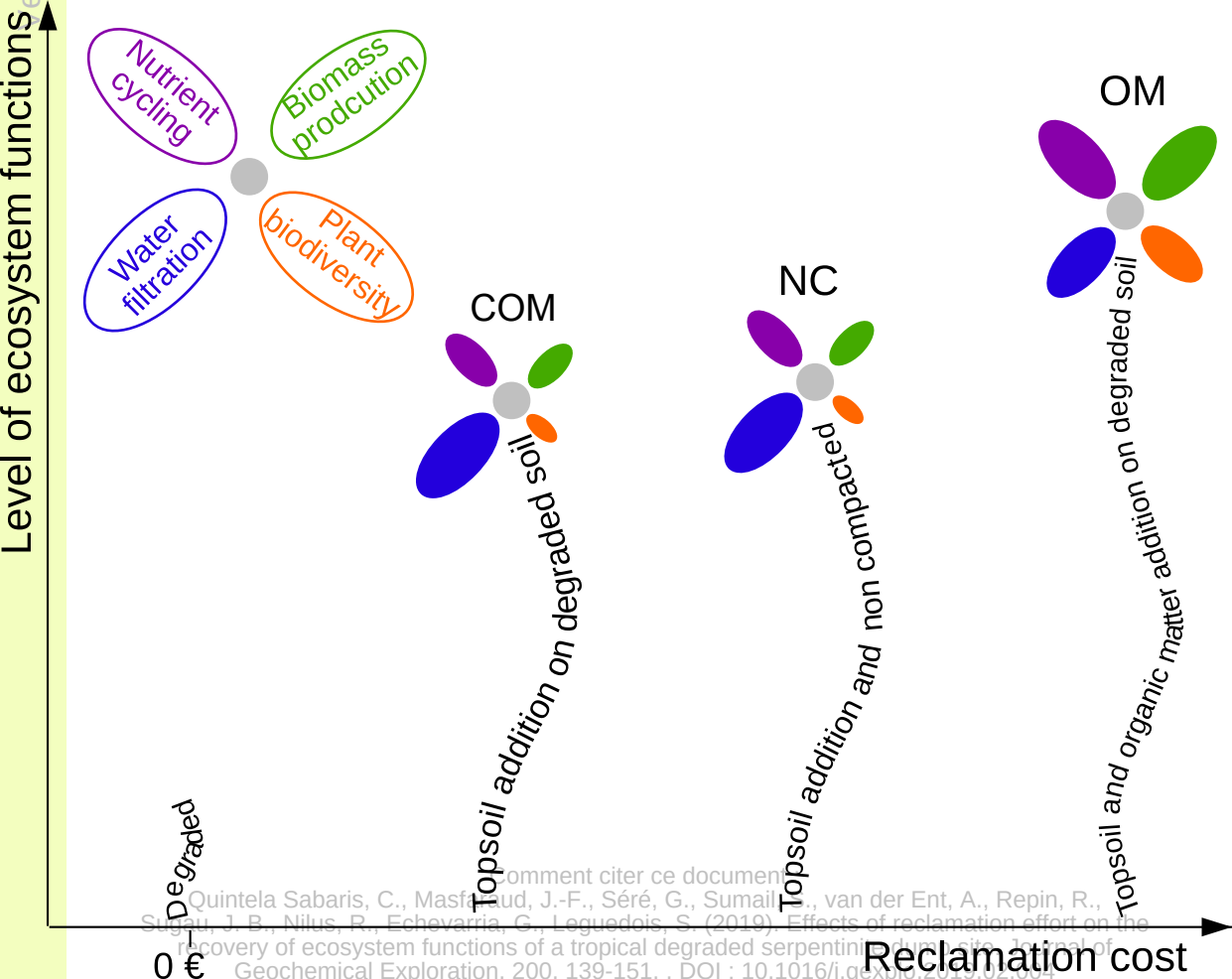
Proposed suggestions and corrections have been included in the new version of the manuscript. Conclusion has been modified to better present the relevance of the obtained results and conclusions.

Highlights:

- A mesocosm experiment was conducted to test the effect of three reclamation treatments on the recovery of selected ecosystem functions in tropical degraded ultramafic areas.
- Measured variables (drainage water fluxes, chemical and biological soil parameters, plant biomass and diversity) were used to develop a multi-criteria analysis.
- Topsoil application + non-compacted + organic amendment produced best results in biomass production and nutrient cycling functions, but increased soluble concentrations and fluxes of Ni (i.e. reduction of water filtration function).

Comment citer ce document :

Quintela Sabaris, C., Masfaraud, J.-F., Séré, G., Sumail, S., van der Ent, A., Repin, R., Sugau, J. B., Nilus, R., Echevarria, G., Leguedois, S. (2019). Effects of reclamation effort on the recovery of ecosystem functions of a tropical degraded serpentinite dump site. *Journal of Geochemical Exploration*. 200. 139-151. . DOI : 10.1016/j.gexplo.2019.02.004



Effects of reclamation effort on the recovery of ecosystem functions of a tropical degraded serpentinite dump site

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Abstract

Surface mining or other disturbing operations on ultramafic outcrops degrade soil conditions and decrease major ecosystem functions in those habitats. Human disturbances exacerbate the harsh conditions of some ultramafic soils, making recovery more difficult. Recovery of ecosystem functions of disturbed areas may vary from passive to active reclamation measures, depending on the starting situation, available resources and expected outcomes. In this study, three treatments (topsoil application, topsoil + non-compacted, and topsoil + non-compacted + organic amendment), representing a gradient of increasing reclamation effort, were tested for the reclamation of a tropical ultramafic Spolic Technosol in Sabah (Malaysia). Soil treatments were tested at the mesocosm-scale for 12 months, and drainage water fluxes, evolution of soil physicochemical parameters, soil microbial activities and spontaneous plant colonisation, diversity and growth were monitored along the duration of the experiment. The results show that application of topsoil improved the plant biomass and water filtration

functions in the non-reclaimed sites, with no additional benefit from lesser compaction. The most intensive treatment (topsoil + non-compacted + organic amendment) gave the best improvements in nutrient cycling and biomass production functions, but with a reduction of water filtration function, due to an increase in soluble concentrations and cumulative fluxes of nickel. Introduced species were dominant in the vegetation colonising the mesocosms, and therefore planting of selected native species will be necessary to create habitats for native biodiversity. This information will be useful to select a strategy for the recovery of degraded tropical serpentinite quarries and dump sites.

Keywords: amendments; ecosystem functions; multi-criteria assessment; soil reclamation; topsoil; tropical ultramafic areas.

1. Introduction

Ultramafic rocks have been exploited as ornamental stones, road-base aggregate, railway track ballast, and are mined to extract metals such as nickel (Ni), chromium (Cr) and cobalt (Co). Surface extractive operations including mining, quarrying, and public engineering (*i.e.* road construction) can degrade soil conditions *via* the compaction and the loss of topsoil, which in turn induces a decrease of major soil ecosystem services, such as limiting erosion, supporting vegetation, and creating a habitat for species (O'Dell and Claassen, 2009). These human disturbances exacerbate the already harsh conditions of many ultramafic areas, *e.g.* the nutrient imbalance and high concentrations of certain metals, making post-operation recovery even more challenging.

Ultramafic outcrops cover approximately 3% of the global terrestrial surface (Guillot and Hattori, 2013), and often host high levels of plant diversity especially in Southeast Asia (van der Ent et al., 2015). Soils derived from ultramafic bedrock are characterised by relatively high concentrations of certain trace elements, including Ni and Co, major cations imbalances with high magnesium to calcium (Mg:Ca) molar quotients) and nutrient deficiencies, especially potassium (K) and phosphorus (P) (Proctor 2003; Galey et al., 2017). Ultramafic soils differ substantial in their physical and chemical characteristics. Soils derived from serpentinite contains both primary minerals (chrysotile, antigorite, lizardite) and secondary minerals (smectites, magnetite, chlorite, talc) (Chardot et al., 2007), and is the most extreme in its chemical properties, with very high Mg:Ca molar quotients, high available Ni and high pH (van der Ent et al., 2017). Leptosols derived from these outcrops are eutrophic with high base saturation and CEC (Echevarria, 2018), and in Sabah hosts a specific and adapted vegetation that tolerates these geochemical peculiarities (van der Ent et al., 2015; 2016).

The recovery of vegetation on ultramafic degraded areas has thus multiple benefits: reduction of metal dispersion, decrease of soil erosion, and support of biodiversity (O'Dell and Claassen, 2009). For the vegetation to re-colonise disturbed ultramafic sites, it is necessary to re-establish the following soil functions: i) nutrient cycling to counteract the lack of essential nutrients; ii) water filtration to limit the dispersion of metals; iii) biodiversity hosting to provide habitat to endemic ultramafic flora; and iv) biomass production to prevent erosion and metal dispersion as well as to promote biodiversity. Reclamation techniques have different

effects on the targeted ecosystem functions. Mineral fertilisers have low effectiveness on revegetation success during recovery of ultramafic areas (O'Dell and Claassen, 2009). Moreover, their application may have undesirable side effects, such as increased mobility and leaching of trace elements (Raous et al., 2010). In contrast, topsoil re-application has been shown to be an effective method to revegetate ultramafic excavated substrates, provided that it is applied at a minimum depth of 30 cm, to allow for adequate rooting depth (O'Dell and Claassen, 2009). Moreover, in extremely anthropised landscapes there is often a lack of appropriate sources of plant germplasm and microbiota (Bradshaw, 2000; Rey Benayas et al., 2008). Topsoil can be used as an inoculum to provide seedbanks and microbiota to ensure the colonisation of disturbed soil (Bradshaw, 2000, 1997). However, this approach requires careful handling of topsoil, as well as stockpiling under appropriate conditions, and fast re-application of topsoil to ensure biological activity (Echevarria and Morel, 2015; Quintela-Sabaris et al., 2018). The use of organic wastes and industrial by-products may also be considered as an amendment for soil construction (Echevarria and Morel, 2015; O'Dell and Claassen, 2009; Séré et al., 2008). Along with nutrients, organic wastes provide soils with other positive effects, such as improvement of the soil structure, increasing cation exchange capacity (CEC), alleviation of hyper-alkaline pH associated with serpentinite minerals, and complexation of trace elements (Bradshaw, 1997).

Recent research to assess the reclamation success of post-operation areas focused on the evaluation of soil physicochemical parameters together with other compartments, such as soil microbiota and plants (Alvarenga et al., 2018) and relating this information with relevant ecosystem services (*e.g.* Burges et al., 2016). In this work on a degraded ultramafic area in Sabah (Malaysia) on the island of Borneo, various levels of intensity of the reclamation treatments were studied. In order to assess the quality of the four targeted ecosystem functions, we monitored drainage water fluxes, the evolution of soil physicochemical parameters, soil microbial activities and spontaneous plant colonisation diversity and growth at the mesocosm scale.

2. Material and Methods

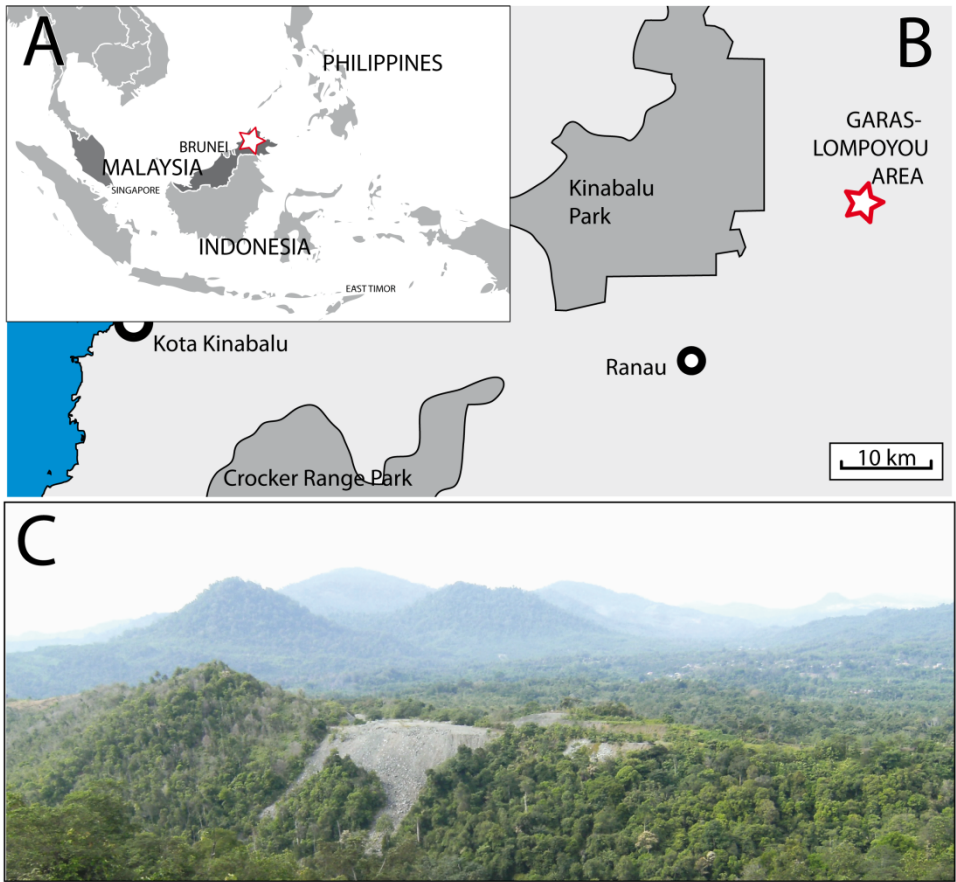
2.1 Studied site

Ultramafic outcrops cover 3500 km² in Sabah, in Malaysia, (Proctor et al., 1988; Repin, 1998) and 151 km² in Kinabalu Park (van der Ent et al., 2015). The study was undertaken at Garas, adjoining the main road from Ranau to Kota Marudu (latitude 6.088° N, longitude 116.798° E), which has a serpentinite outcrop with bare serpentinite soils (Hypereutric Leptosols Hypermagnesian) (van der Ent et al., 2017). These soils have unweathered debris that is rich in clay minerals such as talc and smectite. The soil pH ranges from 6.5 in the soils rich in organic matter to pH 9.8 in the unweathered soil (van der Ent et al., 2017). Civil engineering activities for the enlargement of the road generated several dumpsites in 2008, that currently are poorly vegetated (Figure 1). The climate is tropical with a mean annual temperature of 23°C with low variation (less than 3°C) throughout the year. The annual rainfall is approximately 2,500 mm which is evenly distributed throughout the year. However, two less humid periods in February and August are typical (Aiba and Kitayama, 1999; Kitayama et al., 1998 and Malaysian meteorological department).

2.2 Parent material

Serpentine rock tailings (*i.e.* Spolic Technosols, IUSS Working Group WRB, 2015) were collected from one of the dump sites at Garas. In order to provide the experiment with a starting seed bank, ultramafic topsoil from a disturbed plot with secondary vegetation less than 100 m away from the dumpsite was collected. The plot had a pioneer plant community dominated by the fern *Pteridium esculentum* (Dennstaedtiaceae) and with the presence of different grasses (Poaceae), such as *Imperata cylindrica* and *Miscanthus floridulus*, and pioneer trees (including *Trema* sp. – Cannabaceae, and *Colona* sp. – Malvaceae). Several introduced species, that readily colonise disturbed areas in the region, were also present: *Mimosa pudica* (Fabaceae), *Lantana camara* (Verbenaceae), *Polygala sinensis* (Polygalaceae) and the invasive shrub *Chromolaena odorata* (Asteraceae).

The organic waste was chosen on the basis of its low price and high availability. Malaysia is the second largest world producer of palm-oil, and the cultivation and processing of oil palm (*Elaeis guineensis*) products generates an increasing quantity of liquid and solid organic wastes (more than 51 million tons in 2005, Kala et al., 2009). From the different types of oil palm organic wastes (Singh et al., 2010), we selected dry fronds (*i.e.* oil-palm leaves) as the organic amendment due to significant available stocks in the study area.



134

135 **Figure 1:** Studied area. **A**, regional map. **B**, local map. Dark grey areas mark Malaysia in the regional map, and
136 protected areas of Sabah in the local map. Stars mark the area of study (Lompoyou – Garas) in the North of
137 Borneo, East of Kinabalu Park. **C**, aspect of the study site in Garas (July 2016). Grey non-vegetated serpentinite
138 rocks are prominent in the landscape.

139

140

141 2.3 Experimental setup

142 Sampled material (soils and oil-palm leaves) were transported to Monggis Substation
143 (Kinabalu Park, coordinates latitude 6.200° N, longitude 116.752° E), where the experiment
144 took place. Prior to experimental setup, serpentinite debris was sieved to remove rock
145 fragments >25 cm. Oil-palm leaves were cut in pieces of ~2 cm diameter. Topsoil was
146 thoroughly mixed and sieved <5 mm prior application. These materials were used for the
147 construction of three different soil profiles (Fig. 2): *Compaction and topsoil*, (abbreviated as

COM) which consisted in a soil profile of 40 cm of compacted serpentinite debris –analogous to the current situation of serpentinite dump site at Garas– covered by ~5 cm of topsoil (see section 2.4); *Non-compacted* (abbreviated as NC), which consisted in a soil profile of ~40 cm of serpentinite debris with no compaction applied during filling of mesocosms, plus ~5 cm of topsoil; and *Organic amendment* (abbreviated as OM). This last treatment consisted in a soil profile of 20 cm of non-compacted serpentinite debris overlaid by ~20 cm of serpentinite debris mixed with 30% (volume:volume) oil-palm leaves (Table 1), plus ~5 cm of topsoil. At the bottom of these three different soil profiles, a 5-cm depth sand layer was placed in order to allow drainage of the mesocosms. A piece of geotextile was placed in between sand and serpentinite layers, to avoid particle eluviation. Each treatment was replicated four times. For each treatment, three replicates were studied in medium cylindrical mesocosms (surface 0.12 m², volume of 0.054 m³) and a fourth one was studied in a bigger cylindrical mesocosm (surface 0.20 m², volume 0.088 m³). A drainage system was installed in each mesocosm and connected to 5-L plastic bottles to allow the collection of drainage water. The mesocosms were placed outdoors on a flat surface fully exposed to sunlight and arranged in a randomised complete block design. The experiment started on July 13th 2016 and lasted for 12 months. No watering was applied to the mesocosms for the duration of the experiment, with rain the only water source.

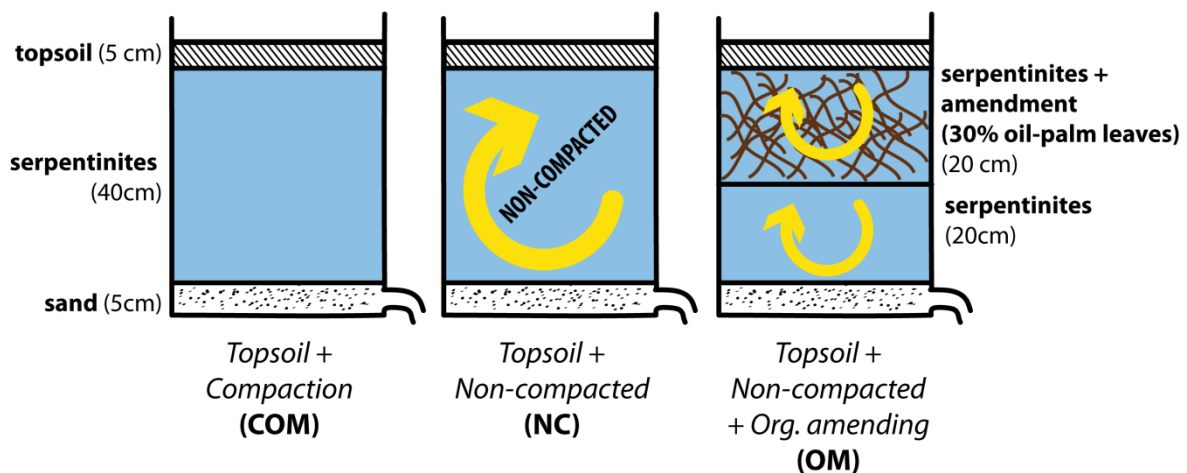


Figure 2: Soil profiles of the three soil treatments tested in mesocosms.

Element	Unit	Mean	SD
C_{org}	g kg ⁻¹	436	3.5
N	g kg ⁻¹	0.78	0.3
C:N		55.9	
Ca	g kg ⁻¹	4.93	0.37
Mg	g kg ⁻¹	2.84	0.36
Ca:Mg		1.74	
K	g kg ⁻¹	4.75	0.48
P	g kg ⁻¹	0.4	0.03
S	mg kg ⁻¹	730	53
Al	mg kg ⁻¹	179	12.6
Fe	mg kg ⁻¹	497	49.9
Mn	mg kg ⁻¹	217	14.5
Na	mg kg ⁻¹	< 162	-
Ni	mg kg ⁻¹	29.1	4.24
Cr	mg kg ⁻¹	9.07	1.19
Co	mg kg ⁻¹	2.78	0.21
Zn	mg kg ⁻¹	< 11	-

169

170 **Table 1:** Composition of oil-palm leaves which were used as organic amendment in OM treatment. Values
171 present the average, followed by SD (n = 3).

172

173 *2.4 Monitoring and sampling*

174 Spontaneous plant colonisation and plant cover was surveyed each two weeks for the duration
175 of the experiment. The monthly volume of drainage water was recorded. One sample (100 ml)
176 of drainage water was collected from each mesocosm every month.

177 At the end of the experiment (on July 2017) each mesocosm was processed as follows: shoots
178 were cut and collected separately for each species. Then, half of the mesocosm was removed
179 to expose the complete soil profile. Soil profile was refreshed until the centre of the pot. A
180 transparent plastic with a 5 × 5 cm grid was placed onto the soil profile to assess rooting depth
181 (Yilmaz et al., 2018). Three soil samples were collected at three depths (0–10 cm, 10–25 cm

and 20–45 cm). All root material from the entire mesocosm was collected as one bulk sample. Plant material was thoroughly washed with tap water and then rinsed with deionised water. Cleaned material was dried until constant weight. Fresh soil samples were sieved to <5 mm and kept at 4° C until analyses.

2.5 Sample analyses

2.5.1 Drainage water

Upon collection, the pH was determined in the water samples. Following, the water samples were filtered (0.45 µm, Pall Acrodisc®) and preserved at 4°C until analysis. Water samples were not acidified in order to measure concentrations of dissolved organic carbon (DOC). However, due to delay in the site access, we were not able to perform this analysis in a decent delay and just have estimates of DOC. The concentrations of Ca, Co, Cr, K, Mg, Na, Ni, P, S and Zn were measured with Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP-AES, Liberty II, Varian Inc, Australia) and expressed as mg of element per L of drained water.

2.5.2 Soils

Fresh soil samples were passed through a 5-mm sieve upon sampling. The activity of soil enzymes alkaline phosphatase, arylsulphatase, β-glucosidase and urease, and the degradation of fluorescein diacetate (FDA) were determined in soil fresh subsamples. The four enzymes analysed are linked to the cycle of essential nutrients in the ecosystem: Carbon (β-glucosidase), Nitrogen (urease). Phosphorus (alkaline phosphatase) and Sulphur (arylsulphatase), whereas FDA degradation is generally considered a proxy for the general hydrolytic activity of the soil (Bandick and Dick, 1999; Adam and Duncan, 2001).

Urease activity was determined using the colorimetric method described by Kandeler and Gerber (1988), and expressed as µg NH₄-N g⁻¹ h⁻¹. Alkaline phosphatase, β-glucosidase and arylsulphatase activities were determined using a microplate protocol and *p*-nitrophenol (*p*NP) linked substrates (Dick et al., 2013). Before incubation, soils were treated with toluene in order to release enzymes inside bacterial cells. Enzyme activity was determined from colorimetric measurements of released *p*NP after incubating soil in a buffered substrate solution (2 h, 37°C), and quantified by reference to calibration curves corresponding to *p*-nitrophenol standards. Enzymatic activities are expressed in µg *p*-NP g⁻¹h⁻¹. Finally,

fluorescein diacetate (FDA) hydrolytic activity of the soil, an indicator of soil biological activity, was measured colorimetrically following the method of (Adam and Duncan, 2001). All values are expressed on an oven-dried soil basis.

Soil subsamples were air-dried and passed through a 2-mm sieve. The soil pH was measured in H₂O using a 1:5 (v/v) ratio. Cation exchange capacity (CEC) was determined colorimetrically after treatment of the soil with a solution of cobaltihexamine trichloride 0.05 N, following the standard method AFNOR NF-X 31-130 (Aran et al., 2008). Exchangeable concentrations of Ca⁺², Mg⁺² and K⁺ in the soil were determined on the filtered soil:cobaltihexamine extracts by ICP-AES (Liberty II, Varian). Total soil C and N measured with a CHNS combustion analyser (Vario Micro Cube, Elementar, Germany). Soil Ni availability was evaluated after extraction with DTPA-TEA (0.005 M DTPA with 0.01 M CaCl₂ and 0.1 M triethanolamine (TEA) at pH 7.3, 1:2 w/v, 2h shaking). Soil subsamples were ground in a ceramic mortar and 0.5 g of dry ground soil were digested in 2 mL of concentrated HNO₃ and 6 mL of concentrated HCl in a hot plate at 105°C. Final solutions were filtered (0.45 µm DigiFILTER, SCP science, Canada) and diluted to 50 mL with deionised water. Pseudo-total soil concentrations of Al, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, P and Zn were measured with ICP-AES (Liberty II, Varian).

2.5.3 Plants

Shoot and root biomass are reported as dry weight (DW) from oven-dried plant samples. After DW was recorded, dry shoots and roots were finely ground using a ball mill. Subsamples (0.5 g) of dry and ground tissue were digested at 95°C in 2.5 mL of concentrated HNO₃ and 5 mL of H₂O₂ (30%). The final solutions were filtered (0.45 µm DigiFILTER) and diluted to 25 mL with deionised water. Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Ni and P concentrations in roots and shoots were measured with ICP-AES (Liberty II, Varian) and expressed in mg g⁻¹ DW plant material. C and N were measured in dry ground roots and shoots using a CHNS analyser (Vario Micro Cube, Elementar, Germany).

2.6 Data analysis

The monthly volume of drained water was divided by surface of mesocosm in order to compare mesocosms of different size. Monthly concentrations of Ca, Cr, K, Mg, Na, Ni, P, S and Zn in drainage water were multiplied by monthly volume of drained water (L m^{-2}) to obtain the monthly elemental flux (g m^{-2}). Differences between treatments in monthly elemental flux were assessed by means of Principal Components Analysis (PCA). Cumulative fluxes of Co, Cr and Ni (main trace elements in ultramafic soils) at harvest were assessed by one-way ANOVA analyses (factor *treatment*).

A PCA analysis was performed on the soil dataset including pH, CEC, soil enzyme activities and total, exchangeable and DTPA-extractable concentrations of several elements. Phosphorus was excluded from the analysis because all samples collected at 10–20 and 20–40 cm depth had P concentrations below quantification limit (approx. 100 mg kg^{-1}). Selected soil variables were further explored by blocked two-way ANOVAs (factors *treatment* and *depth*). Plant diversity in each mesocosm was calculated using Shannon's diversity index (H'). This index was computed with the following formula: $H' = -\sum p_i \ln(p_i)$. Where i corresponds to each species in the vessel and p_i is the proportional abundance of species i . Differences in plant species composition between treatments were assessed by 2D Non-Metric Multidimensional Scaling (NMDS). In both cases, shoot biomass data was used as a proxy for the abundance of each species. H' was computed with function *diversity*, and NMDS was computed using function *metaMDS*, both of them from package *vegan* for R. The time until the detection of the first plant growing on each mesocosm was registered. Time data were used to assess the effects of treatment on spontaneous plant colonisation. Between-treatment differences in time to the first colonising plant, in plant diversity and root and shoot biomass at harvest were assessed by blocked one-way ANOVA (factor *treatment*), whereas differences in vertical root distribution at harvest were assessed by blocked two-way ANOVA (factors *depth* and *treatment*). A PCA analysis was performed on plant chemical data (i.e. total root and shoot concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, N, Ni and P). These data were further explored by blocked two-way ANOVA (factors *fraction* –root vs shoot– and *treatment*). PCAs and ANOVA were performed in SPSS (v. 15, SPSS Inc., Chicago, IL, USA).

272 A multi-criteria assessment of treatment effects on four ecosystem functions (biomass
 273 production, nutrient cycling, water filtration and plant biodiversity habitat) has been
 274 developed. For that, several of the measured variables have been selected and grouped as
 275 indicators for each function (Table 2). The values of each indicator were ranked in four
 276 classes (0, +, ++, +++) and computed an average rank for each treatment and ecosystem
 277 function. Trace elements and nutrient concentrations in soils were chosen as explanatory
 278 variables to aid in the interpretation of treatment results.

279

Functions	Indicators
Biomass production	Aerial dry biomass
	Root dry biomass
	Plant cover
Nutrient cycling	Total cumulative fluxes of K, Ca, Na, P, S
	Nutrient shoot concentrations (C, N, P, K, S, Ca)
	Nutrient root concentrations (C, N, P, K, S, Ca)
	Enzyme activities and FDA (at 20 cm)
	Root abundance at 20 cm depth
Water filtration	Total cumulative fluxes of Ni, Cr, Co, Zn
	Maximal concentration of Ni, Cr, Co, Zn
	Exceedance of critical limits
Plant biodiversity habitat	Number of species
	Shannon's Index
	Presence of introduced species (N of species and % of total shoot biomass)
Explanatory variables	Nutrient and metal (total and available) concentrations in soils

280

281 **Table 2:** Selected indicators for the determination of four ecosystem functions in the experiment. Explanatory
 282 variables for the treatment results are presented in last row.

3. Results

3.1 Evolution of drainage water

Accumulated precipitation for the 12 months duration of the experiment nearly reached 3000 mm. The accumulated volume of drained water from the mesocosms was ~2073 mm per mesocosm ($\pm$ 393 mm), with no difference between the treatments (one-way ANOVA, p = 0.799). PCA analysis of the drainage water yielded three Principal Components (PCs) that explained 71% of total variance of drainage water composition. PC1 (30% of variance) was mainly determined by fluxes of Ca, Na and S; whereas PC2 (28% of variance) was related to fluxes of K, Mg and Ni. Cr and P reflect on PC3 (13% of variance) (Fig. 3A). Considering first two PCs, each group of variables had different behaviour: Ca, Na and S fluxes (PC1) were *time dependent* (*i.e.* a higher flux at the beginning of the experiment and fluxes reduced with time, Fig. 3B). Conversely, K, Mg and Ni fluxes were independent of time but more related to the *volume of drainage water* (*i.e.* linked with the volumes of drainage water, Figure 3B). The position of samples on the two PCs shows that COM and NC treatments follow similar trends: they vary along PC1 but, for almost all samples, they stay in negative values of PC2 (*i.e.* lower fluxes of K, Mg and Ni). OM samples have similar variation along PC1, but have positive values in PC2, which indicates increased flux of K, Mg and Ni (Fig. 3). All the treatments had maximum values in PC1 during first three months, coinciding with the highest concentrations of Ca, Na and S in the drainage water.

The three treatments had contrasting effects on the soluble concentrations of Cr and Ni. Co concentrations were always below the limit of quantification (0.002 mg L^{-1}). In the case of Cr, OM treatment reduced soluble concentrations from 0.01 mg L^{-1} (treatment COM) to 0.005 mg L^{-1} (treatment OM, Fig. 4A). However, all water samples had Cr concentrations lower than the EU threshold for Cr in drinking water (*i.e.* 0.05 mg L^{-1} , Council Directive 98/83/EC). The organic amendment has the opposite effect on Ni: soluble concentrations of this element increased in the OM treatment (0.038 mg L^{-1}) with respect to other two treatments (0.007 mg L^{-1} , Fig. 4A). Thus, most of the water samples from OM treatment exceeded the EU threshold for Ni in drinking water (*i.e.* 0.02 mg L^{-1} , Council Directive 98/83/EC).

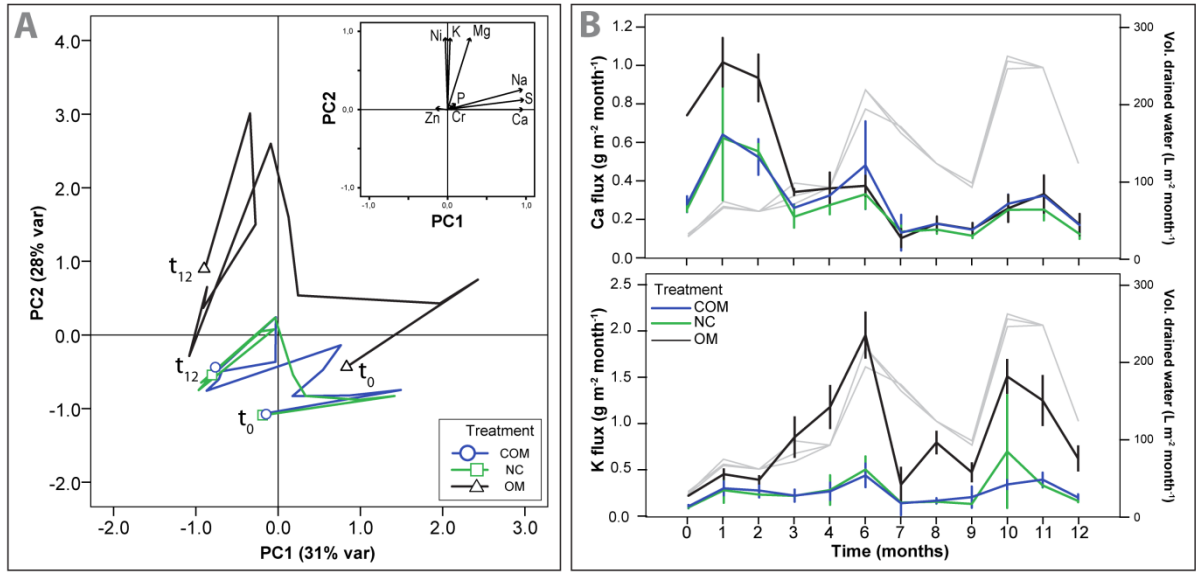


Figure 3: A PCA analysis of elemental flux in drainage water. Different lines present the evolution of monthly fluxes for each treatment. Circles, squares and triangles mark elemental flux at $t = 0$ (July 2016) and at $t = 12$ (July 2017) for each treatment. Contribution of each measured variable to PCs is presented in the inset. **B** Evolution of monthly elemental flux for Ca (top) and K (bottom) along the experiment ($\text{g m}^{-2} \text{ month}^{-1}$). Different colours correspond to average values for each treatment ($\pm \text{SD}$): blue , COM; green , NC; black , OM. Grey lines present the monthly volumes of drained water ($\text{L m}^{-2} \text{ month}^{-1}$), averaged for each treatment.

As a result of the variation in soluble concentrations of Cr and Ni, a variation in cumulative flux of these elements was observed. In the case of Cr, the OM treatment reduced accumulated flux to about half of the other treatments (0.07 g m^{-2} in OM vs. 0.15 g m^{-2} in COM and NC, Fig. 4B). In the case of Ni, there was an opposite trend: Ni accumulated flux increased up to nearly 0.06 g m^{-2} in the OM treatment, whereas it was approximately 0.01 g m^{-2} in other two treatments (Fig. 4B).

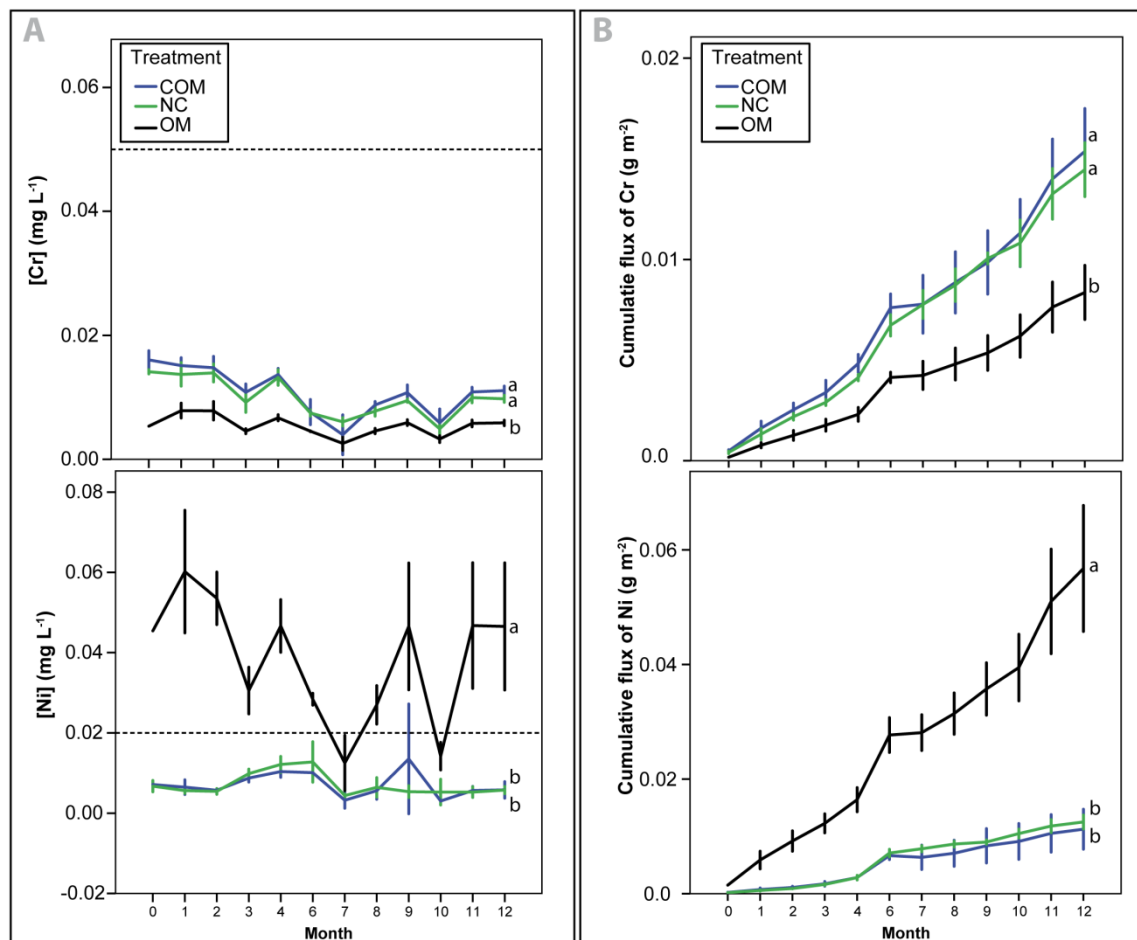


Figure 4: Variation in Cr and Ni soluble concentrations (A) and cumulative element flux (B). Dashed horizontal lines in 5A mark the EU threshold for Cr and Ni in drinking water. Different letters mark significant differences ($p < 0.05$) between *treatments* (posthoc analysis with Bonferroni correction). Different colours correspond to average values for each treatment ($\pm$ SD): blue, COM; green, NC; black, OM.

3.2 Soil parameters

During experiment set-up, we applied a 5-cm thick layer of topsoil on the three tested treatments. Water infiltration and root development contributed to the mixing of topsoil with the underneath serpentinite debris. Thus, at harvest, we observed in all treatments a top 10-cm thick reddish-coloured layer contrasting with the deeper grey-blue soil layers.

A PCA was performed on soil chemical and biological parameters. Three PCs were inferred, explaining 92% of total variance in the soil parameters. The PC1 alone accounted for most of the variance (85%). Variables with positive loadings in this PC included all measures of

biological activity, pseudototal Al, Ca, K, C and N concentrations, CEC and exchangeable Ca and Ni, and DTPA-extractable concentrations of Co and Ni. In contrast, pH, total concentrations of Fe, Mg and trace elements (Co, Cu, Mn, Ni and Zn) and exchangeable Mg had negative loadings on PC1. PC2, which explained 5% of variance only, was contributed by total Cr concentration and exchangeable K (Figure 5A). Projection of soil samples on two first PCs showed a clear differentiation between soil depths. Samples of the upper part of the soil profile (which correspond to contact between topsoil – similarly in the three treatments– and serpentinite debris) were placed in positive values for PC1, and near 0 for PC2 (Fig. 5B). Thus, these samples had the best chemical and biological fertility parameters among all the analysed soils (*i.e.* higher CEC, C, N, Ca and K concentrations, higher enzyme activities). In contrast, samples of the middle (10-25 cm depth) and the bottom (25-45 cm depth) of the soil profile in treatments COM and NC were plotted in the most negative values of PC1 (*i.e.* higher pH, exchangeable Mg and total concentrations of Mg and trace elements, Fig 5). Interestingly, samples of 10-25 cm – where oil-palm leaves were applied – and 25-45 cm of OM treatment were placed in less-negative values of PC1 than samples from other treatments at same depths, and also had the most positive values in PC2 (*i.e.* better chemical fertility parameters compared to other two treatments and higher Cr and exchangeable K).

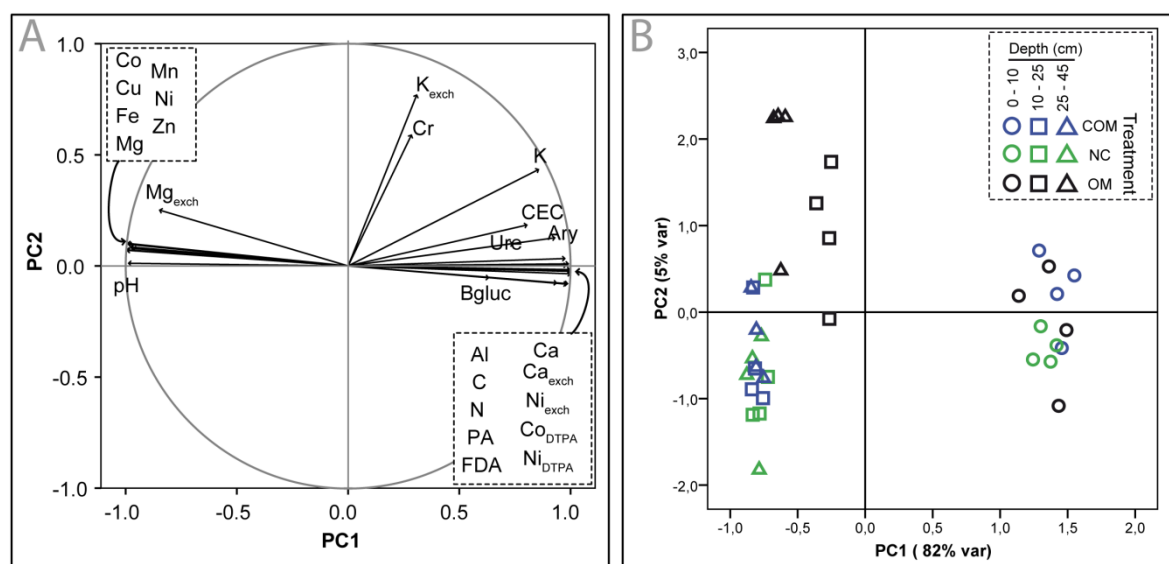
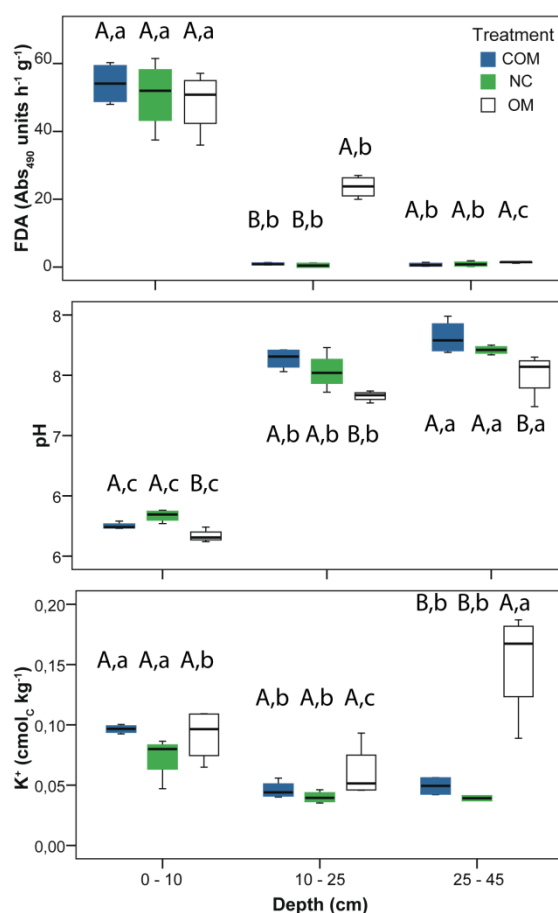


Figure 5: PCA of soil parameters. **(A)** Loadings of each soil variable in two first Principal Components (PCs). **(B)** Projection of soil samples (each symbol indicates one sample) on first two PCs. Different colours correspond

362 to each treatment (blue, COM; green, NC; black, OM), whereas different symbols mark depth (circles, 0-10 cm;
 363 squares, 10-25 cm; triangles, 25-45 cm). Concentrations of different elements are abbreviated as their chemical
 364 symbols (plus $DTPA$ and $_{exch}$ sub indices in DTPA-extractable and exchangeable concentrations, respectively) in
 365 (A), whereas soil enzymes were abbreviated as follows: Ary, arylsulphatase; Bgluc, β -glucosidase; FDA,
 366 fluorescein diacetate degradation; PA, alkaline phosphatase; Ure, urease.

367

368 The patterns unveiled by soil PCA were further explored by ANOVAs. Three soil variables
 369 related to PCs (FDA degradation, positive contribution to PC1; pH, negative contribution to
 370 PC1; and K exchangeable concentration, positive contribution to PC2; Fig. 6) were compared.
 371 In the three cases, COM and NC had similar values, whereas treatment OM had a different
 372 response. Organic matter addition significantly increased FDA degradation at intermediate
 373 depth; reduced pH at three depths considered and increased exchangeable K concentration at
 374 25-45 cm depth in the profile (Fig. 6).



375

Figure 6: Differences in soil parameters related to two first Principal Components of soils PCA (FDA degradation, pH and exchangeable K concentration) between different treatments and soil depths. Each treatment is indicated by a different colour (blue, COM; green, NC; black, OM) whereas depth is indicated along the horizontal axis. In each plot, different capital letters indicate significant differences between *treatments* within each *depth* and different lower case letters denote significant differences across all *depths* within each *treatment*.

3.3 Plant diversity and other plant parameters

A total of 17 different plant species colonised the 12 mesocosms. The number of species per mesocosm ranked from 3 to 14. Eight species were present in just one mesocosm. Two introduced species were present in the majority of pots: *Chromolaena odorata* (12 pots) and *Polygala sinensis* (10 pots) (Table 3). Plant communities were similar in the three treatments, as indicated by the partially-overlying areas in the NMDS graph (Fig. S1, supplementary material).

Species code	Species name	Plant family	N of mesocosms
CHOD *	<i>Chromolaena odorata</i>	Asteraceae	12
POSI *	<i>Polygala sinensis</i>	Polygalaceae	11
SCSP	<i>Schoenoplectus sp.</i>	Cyperaceae	10
G#01	Grass01	Poaceae	7
PASP	<i>Paspalum sp.</i>	Poaceae	6
NEGI	<i>Neonauclea gigantea</i>	Rubiaceae	4
CASP	<i>Carex sp.</i>	Cyperaceae	3
PACO *	<i>Paspalum conjugatum</i>	Poaceae	3
BLBA	<i>Blumea balsamifera</i>	Asteraceae	2
CYSP	<i>Cyperus sp.</i>	Cyperaceae	1
TRSP	<i>Trema sp.</i>	Cannabaceae	1
AS#01	Asteraceae 01	Asteraceae	1
F#01	Herbaceous Forb 01	? (Dicot)	1
F#02	Herbaceous Forb 02	? (Dicot)	1
S#01	Shrub 01	? (Dicot)	1
T#01	Tree01	? (Dicot)	1
T#02	Tree02	? (Dicot)	1

Table 3: List of species colonising ultramafic mesocosms. For each species the more precise identification is provided. Asterisks (*) next to species code indicate alien species. Last column indicates the number of mesocosms where each species was found.

With regard to the plant diversity and biomass, significant differences were only found in shoot biomass (Table 4). For this parameter, the OM treatment yielded higher shoot biomass (average of 33.6 g per mesocosm) than any of the other two treatments, and nearly triplicated biomass values compared to COM. Shoot: root ratio differed among treatments. Treatment OM had the highest value (2.56), whereas the lower was in treatment COM (1.54). There were no significant differences in plant cover between treatments. Cover values ranked from 66.2% (average for treatment NC) and 91.4% (average for treatment OM).

Treatment	N sp	Shannon H'	Shoot biomass (g)	Root biomass (g)	Shoot:Root ratio	Plant cover (%)
COM	4.5 ± 1.3	1.03 ± 0.37	13.9 ^b ± 3.0	9.2 ± 2.0	1.54 ± 0.29 ^b	77.9 ± 11.5
NC	4.7 ± 1.0	0.82 ± 0.45	19.4 ^{ab} ± 7.8	12.4 ± 5.2	1.58 ± 0.28 ^{ab}	66.2 ± 23.3
OM	7.2 ± 4.6	1.04 ± 0.28	33.6 ^a ± 14.6	13.6 ± 6.5	2.56 ± 0.57 ^a	91.4 ± 6.5
Grand mean (12 mesocosms)	5.5	0.96	22.3	11.7	1.89	78.5

Table 4: Summary of plant diversity and biomass in soil mesocosms, and plant cover at harvest. Mean and SD values are presented for each variable. Different superscript letters indicate significant differences between treatments ($p < 0.05$).

The number of plants growing in the mesocosm varied in different ways between the treatments, although there was no significant difference between the treatments at harvest (Fig. 7). However, there were significant differences in the time to the appearance of the first colonising plant. In COM, colonisation was faster than other two treatments, average time for the first coloniser was 45 days. The number of plants peaked at around 200 days from the start of experiment. However, plant mortality (that affected especially the seedlings) reduced progressively the number of individuals, and around day 300 there were (on average) fewer than 25 plants per mesocosm. The first coloniser in treatment NC appeared (on average) at day 77. Plant colonisation was low along all the experiment, but during the last month the number of plants strongly increased to mean values higher than 75 plants per mesocosm. The plant colonisation in treatment OM was delayed until day 108 and followed a similar pattern

as treatment NC during first half of the experiment. Around day 250 there was an important increase in the number of plants per mesocosm. After a strong decrease in the number of plants around day 300, there was a fast increase until values higher than 50 plants per mesocosm.

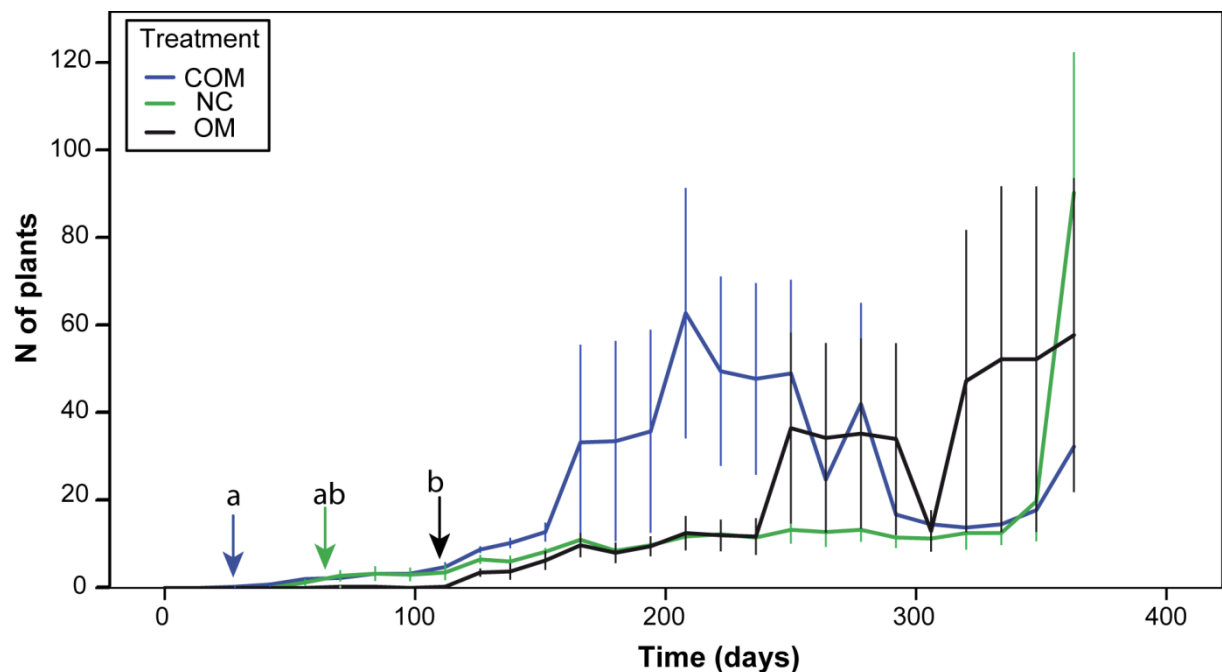


Figure 7: Evolution of plant colonisation of mesocosms. Lines indicate the average number of plants ($\pm$ SD) per treatment (blue, COM; green, NC; black, OM) at each number of days from the beginning of the experiment. Vertical arrows mark for each treatment the average number of days to the detection of the first coloniser plant in a mesocosm, with different superscripts marking significant differences ($p < 0.05$) between treatments.

Despite of the lack of differences in root biomass, we found significant differences in vertical root distribution along soil profile. The three treatments had similar root abundances down to 15 cm deep. At increasing depths, root abundance decreased for treatments COM and NC whereas in OM treatment root abundance was higher than 50% until 35 cm deep (Fig. 8).

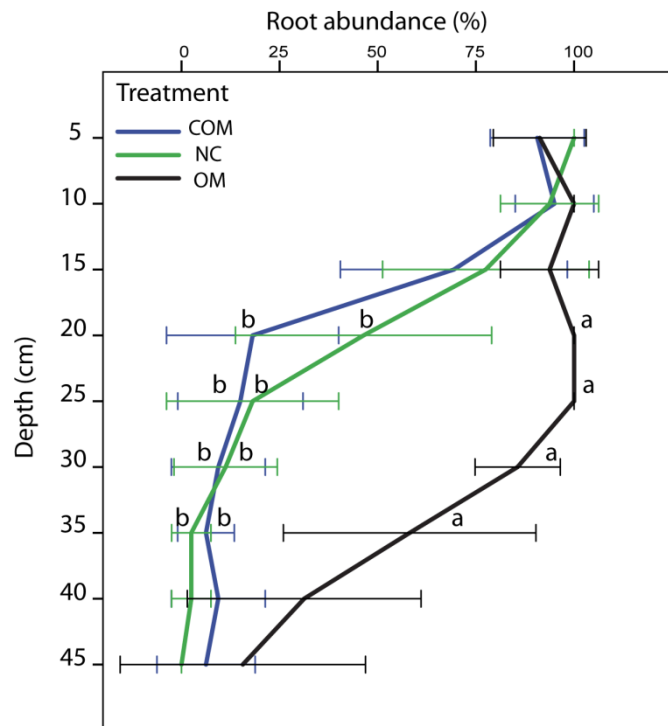


Figure 8: Vertical distribution of root abundance in the studied mesocosms. Different colours correspond to different treatments (blue, COM; green, NC; black, OM). Different lower-case letters mark significant differences between *treatments* for each *depth*.

PCA analysis on concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, N, Ni and P in different *fractions* of plant tissues (either roots or shoots) revealed three PCs that explained more than 81% of total variance in plant tissue composition. The concentrations of Fe, Mn, Cu Co, Cr and Ni had positive contribution to PC1 (49% of total variance); whereas N, K and Mg had positive contributions for PC2 (21% of total variance). P had an intermediate contribution to both PCs (Fig. 9A). Ca had only influence on PC3 (11% of variance). The projection of plant samples on first two PCs showed a clear separation between plant fractions (roots and shoots) along PC1 (Fig. 9B). This suggests that higher concentrations of trace elements are present preferentially in roots. Two-way ANOVAs (factors *treatment* and *fraction*) were performed on elements related to PCs (Ni, Cr, Cu and P to PC1; K, Mg, N and P to PC2, Ca to PC3) (Fig. 10). No interactions were observed between the *treatment* and *fraction* (*i.e.* concentrations of each element had the same pattern of variation across treatments in roots and in shoots). Irrespective of treatments, P and K concentrations were higher in the shoots

than in the roots, whereas Cu, Cr and Ni were higher in the roots than in the shoots. Ca, Mg and N concentrations were similar in the roots and in the shoots. The plants from OM treatment had lower concentrations of Ca, Cr and Ni than other treatments, whereas K concentration in the roots and in the shoots was the highest (on average 8270 $\mu\text{g g}^{-1}$) in the plants from the OM treatment (Fig. 10).

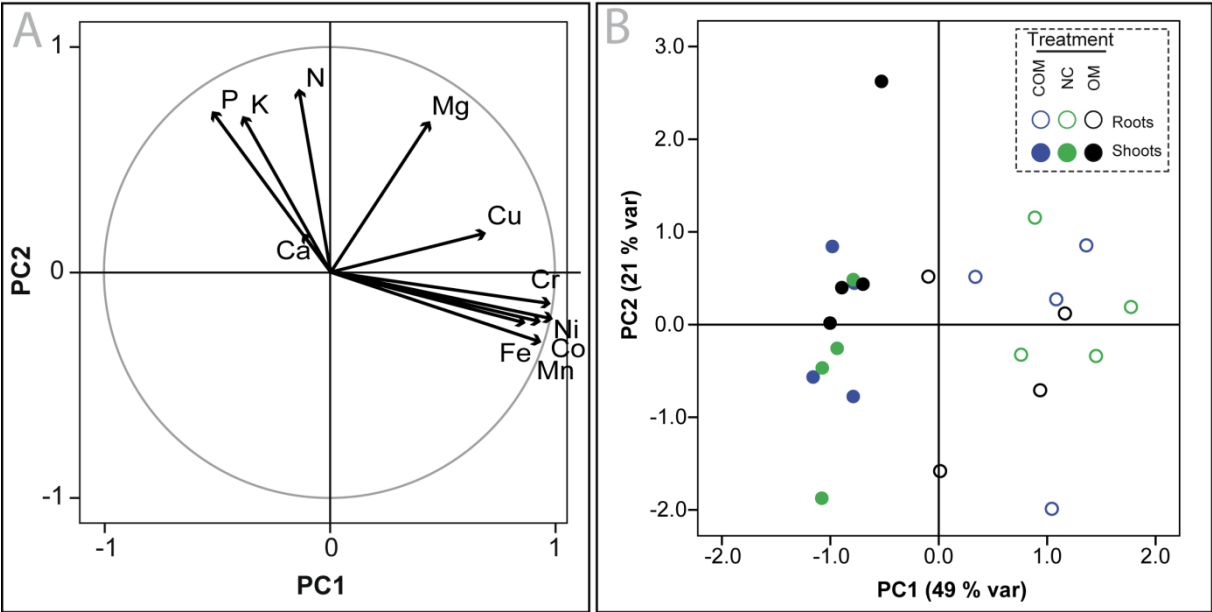


Figure 9: PCA of plant samples. **(A)** Loadings of each variable in two first Principal Components (PCs). **(B)** Projection of plant samples (each dot indicates one sample) on first two PCs. Different colours correspond to each treatment (blue, COM; green, NC; black, OM), whereas filled symbols indicate shoot samples and empty symbols indicate roots. Concentrations of different elements are abbreviated as their chemical symbols in (A).

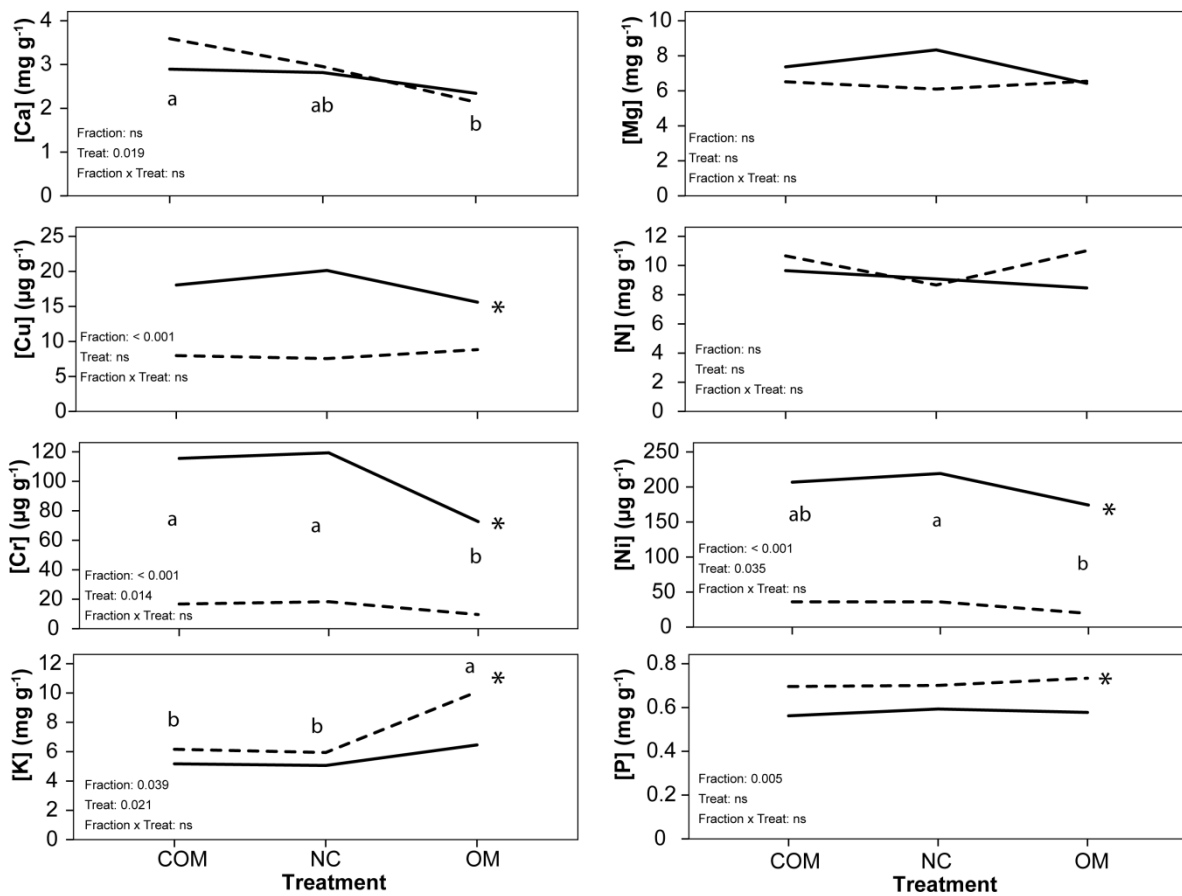


Figure 10. Variation in plant chemical composition. Each graph presents average concentration for each element in roots (continuous line) and shoots (dashed line) across three tested treatments. ANOVA results are presented in each graph as the p-values of the effects of the factors *treatment*, *fraction* (roots or shoots) and interaction *treatment* × *fraction*. ns: non-significant (p-value > 0.05). Asterisks mark significant differences among *fractions* irrespective of *treatments*. Different lower-case letters mark differences between *treatments*, irrespective of plant *fractions*.

Functions	Relevant indicators	Indicator values					Average rank			
		TOP	NC	OM	CON	UND	TOP	NC	OM	CON
Biomass production	Aerial dry biomass (g)	13.9 ± 3.0	19.4 ± 7.8	33.6 ± 14.6	0	nd	++	++	+++	0
	Plant cover (%)	77.9 ± 11.5	66.2 ± 23.3	91.4 ± 6.5	0	100				
Nutrient cycling	K total cumulative flux (g m ⁻²)	3.2 ± 0.7	3.6 ± 0.7	10.8 ± 2.1	nd	nd	++	++	+++	+
	K shoot concentration (mg g ⁻¹)	6.2 ± 0.8	5.9 ± 1.8	10.1 ± 3.6	0.0	6.7 ± 2.5				
	FDA hydrolysis at 20 cm depth (Abs490 units g ⁻¹ h ⁻¹)	0.9 ± 0.3	0.5 ± 0.6	23.7 ± 3.2	0.8 ± 0.7	77.1 ± 26.0				
	Alkaline Phosphatase activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	10.0 ± 7.5	20.1 ± 8.0	24.9 ± 6.8	13.1 ± 5.5	156.1 ± 52.9				
	Arylsulphatase activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	1.1 ± 0.6	2.4 ± 1.7	23.4 ± 4.8	1.8 ± 2.0	59.9 ± 21.7				
	β-glucosidase activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	18.6 ± 5.8	14.8 ± 7.6	14.5 ± 3.5	34.3 ± 19.3	62.2 ± 17.6				
	Urease activity at 20 cm depth (µg p-NP g ⁻¹ h ⁻¹)	0.6 ± 0.4	0.4 ± 0.5	3.4 ± 2.0	2.0 ± 1.7	6.4 ± 3.3				
	Root abundance at 20 cm depth (%)	18.1 ± 21.9	46.4 ± 32.6	100 ± 0.0	0	nd				
Water filtration	Ni cumulative flux (mg m ⁻²)	11.3 ± 3.5	12.5 ± 1.2	56.8 ± 11.0	58.0	nd	+++	+++	++	+
	Ni maximum leachate concentration (µg L ⁻¹)	34	20	81	250	nd				
	Zn maximum leachate concentration (µg L ⁻¹)	7	126	6	nd	nd				
	Exceedance of Ni critical limits (number of times)	1	1	36	13	nd				
	Exceedance of Cr critical limits (number of times)	0	0	0	13	nd				
	Exceedance of Zn critical limits (number of times)	0	0	0	nd	nd				
Plant biodiversity habitat	Number of plant species	4.5 ± 1.3	4.8 ± 1.0	7.2 ± 4.6	0	6 ± 1.6	+	+	++	0
	Presence of exotic species (% of total shoot biomass)	66.6 ± 18.0	66.3 ± 25.8	70.1 ± 25.4	0	0				

471 *nd*: not determined

Table 5: Data used for the multi-criteria assessment of reclamation treatment effects. First column indicates the four ecosystem functions important for the reclamation of ultramafic dumpsites. Second column presents selected indicators that discriminate among treatments (and the units within brackets), followed by three columns with the average values ($\pm$ S.D.) of each indicator averaged for each treatment (COM, topsoil application + compaction; NC, topsoil + non-compacted; OM, topsoil + non-compacted + organic amendment), a fourth column (CON, control) showing the values in non-reclaimed tropical ultramafic spoils (data recovery from Raous et al. 2011 and Auber, 2017 *unpublished*) and a fifth column (UND, undisturbed) showing values for some parameters in an undisturbed ultramafic soil (five 4-m² square plots from mature *Ceuthostoma terminale* forest inside Kinabalu Park; Quintela-Sabaris, 2018 *unpublished*). Last four columns indicate the average ranks for each treatment and for each ecosystem function.

4. Discussion

Heavily disturbed soils, such as those in the study site, have reduced potential to provide ecosystem services because of the loss of soil functions (Echevarria and Morel, 2015). The recovery of ecosystem functions of disturbed areas may vary from passive to active reclamation measures, depending on starting situation and expected outcomes (Bradshaw, 2000; Rey Benayas et al., 2008).

In this study, three treatments, representative of realistic practices were tested (topsoil + compaction – COM –, topsoil + non-compacted –NC–, and topsoil + non-compacted + organic amendment – OM –) for the reclamation of a tropical Spolic Technosol on serpentinite. These three treatments can be ordered in a gradient of increasing reclamation effort, which implies inevitably increasing economic cost.

4.1 Biomass production

The establishment and increase of plant cover assist in the stabilisation of dump site soils, reducing the effect of wind and rain erosion (Tordoff et al., 2000), thereby improving ecosystem functions. After 12 months of the experiment, the plant cover was similar in the three treatments (average cover 78.5%), but it almost doubled observed plant cover in eight non-reclaimed ultramafic dumpsites and quarries in Sabah (48.3%, Quintela-Sabaris et al., *unpublished*).

505 The lack of differences in plant cover between treatments can be explained by the
506 beneficial effects of topsoil, which was applied to the three treatments. Topsoil
507 application is known to improve plant colonisation and growth in post-operation
508 environments (*e.g.* Echevarria and Morel, 2015; Losfeld et al., 2015; O'Dell and
509 Claassen, 2009). Indeed, 30 cm of topsoil is recommended to provide plants with
510 sufficient rooting depth (O'Dell and Claassen, 2009). In this study, we expected
511 that the main effects of the thin topsoil layer (just 5 cm) was acting as seed bank,
512 source of microbiota, and seedbed. However, topsoil application also improved
513 soil chemical and biological fertility parameters in the top 10 cm of soil profile, in
514 comparison with the deeper soil layers. Root development in the COM and NC
515 treatments was restricted to this nutrient rich layer, but in the OM treatment the
516 roots grew well (root abundance >50%) in the layer where oil palm fronds have
517 been applied. Despite of these differences in the vertical distribution, the root
518 biomass was similar between the treatments, whereas an increase in shoot biomass
519 (1.5 to 2 times higher than in the other two treatments) was observed in the OM
520 treatment.

521 The model of 'functional equilibrium' (Brouwer, 1962) states that plants allocate
522 more biomass towards shoots or roots to capture more of those resources that most
523 strongly limit plant growth. According to this model, it has been shown that plants
524 respond to a decrease in below-ground resources with increased allocation to roots
525 (*i.e.* decreased shoot:root biomass ratio) (Poorter and Nagel, 2000). Shoot:root
526 biomass ratio was higher in the OM treatment than in the other two treatments.
527 Thus, we can interpret that plants in COM and NC treatments, growing on nutrient
528 poor soils, allocated more biomass to roots to capture resources to grow; whereas
529 plants in OM treatment, as a consequence of the organic amendment, were less
530 limited by nutrients and could allocate resources to shoot biomass during growth.
531 In summary, topsoil application has fulfilled a major role in the restoration of plant
532 cover and biomass production, and organic amendment has provided additional
533 nutrients that reduced the nutrient limitations in the serpentinite soil.

534

535 4.2 Nutrient cycling

536 Towards the top and bottom of the soil profile, variables indicative of nutrient
537 cycling were similar between the treatments. At 10–25 cm depth, the main
538 differences were between the OM treatment and the other two treatments. Organic
539 amendment with oil palm fronds increased the soil total concentrations of N, Ca
540 and K (P and S were below the limit of quantification in all of the treatments).
541 However, the cycling of K and S appears to be most affected by organic
542 amendment: the cumulative flux and the concentration of K in roots and the root
543 and shoot S concentrations were higher in OM treatment than in the other two
544 treatments; whereas C and Ca concentrations were higher in COM and NC
545 treatments. Rooting depth increased greatly in OM treatment. This makes
546 vegetation less affected by drought and allows plants to explore more soil volume
547 for nutrient scavenging. Nevertheless, the most remarkable difference between
548 OM treatment and other two treatments in nutrients cycles was visible in the
549 improvement of bacterial activities in soil after application of organic
550 amendments: FDA hydrolysis (indicative of soil hydrolytic capacity) and enzyme
551 activities related to the cycling of important nutrients: nitrogen (urease),
552 phosphorus (alkaline phosphatase) and sulphur (arylsulphatase) were higher in OM
553 treatment. This effect has been observed in other metal-rich organic-amended soils
554 (*e.g.* Touceda-González et al., 2017) or degraded agricultural soils (*e.g.* Ros et al.,
555 2003) and can be attributable to the direct effect of the organic amendment or to
556 indirect effect *via* the increased presence of roots (and thus induced biological
557 activity due to root exudation) at that depth.

558

559 4.3 Water filtration

560 Ultramafic mine spoils may cause problems to groundwater pollution due to
561 leaching of Ni and Cr (Raous et al., 2010). Organic amendments have been widely
562 used on the reclamation of mine soils to reduce metal mobility and bioavailability
563 (*e.g.* Alvarenga et al., 2009a, 2009b; Burges et al., 2016). In this study , the OM
564 treatment reduced Cr soluble concentrations and cumulative flux, but induced an
565 increase of both parameters for Ni and the exceedance of the EU Ni-thresholds for

drinking water. Two processes may explain this unexpected effect of organic amendment on Ni mobility: i) increased mobility of Ni due to slight pH reduction (around 0.5 points); and, ii) the formation of soluble complexes of Ni with organic compounds either released during degradation of labile fraction of the organic amendment (Kala et al., 2009) or exuded by roots (Clemente et al., 2010). We observed that drainage water samples from OM treatment had a dissolved organic carbon concentration twice that of the other two treatments (data not shown). This suggests that the second explanation is the most probable. It would be interesting to develop a similar analysis using composted oil palm wastes (*i.e.* stabilised organic matter) to determine which is the source of Ni-solubilising organic compounds.

4.4 Plant biodiversity habitat

Spontaneous colonisation of post-mining sites has shown similar success as technical reclamation (Bradshaw, 2000; Mudrák, O. et al., 2010; Šebelíková et al., 2016). In some cases, plant communities recovered by spontaneous revegetation were more diverse than reclaimed sites (Šebelíková et al., 2016). After one year of the experiment, plant communities growing on the mesocosms had many similarities with the site where topsoil was collected. However, three introduced species (especially *Chromolaena odorata*) were dominant: on average 68% of shoot biomass corresponded to introduced species across all treatments. Although it is difficult to predict the evolution of plant communities from the results of one year of colonisation, the results suggest that planting of native species that can outcompete the invasive species is needed to meet a minimum objective for creating a habitat for native plant biodiversity. The study of functional traits of pioneer plant species on metal-rich disturbed habitats may help in the selection of interesting species for reclamation (Ilunga wa Ilunga et al., 2015). Ongoing research in tropical ultramafic areas on this topic points to perennial species with shallow roots and lateral spreading capacity as the ideal candidates (*Quintela-Sabaris et al. unpublished*). Grasses and sedges such as *Imperata cylindrica*,

596 sedges such as *Fimbristylis* sp. (Cyperaceae) or the fern *Pteridium esculentum*
597 appear to be good choices.

598

599 4.5 Global assessment of reclamation treatments on ecosystem functions

600 We tested three treatments, which represent increasing reclamation effort, and
601 their effects on four ecosystem functions were assessed, considering variables
602 from multiple compartments of the ecosystem. Our multi-criteria approach (see
603 table 5) indicates that the less intensive approach (compaction + topsoil) improved
604 plant biomass and water filtration functions with respect to non-reclaimed sites. As
605 noted above, the benefits of topsoil application have been demonstrated elsewhere
606 (Echevarria and Morel, 2015; Losfeld et al., 2015; O'Dell and Claassen, 2009).
607 Moreover, additional reduced compaction efforts did not lead to additional benefits
608 compared to topsoil application. In contrast, the most intensive treatment (non-
609 compacted + organic amendment + application of topsoil) had the greatest
610 improvements on nutrient cycling and biomass production functions, but at the
611 same time the performance of this treatment on water filtration was lower than the
612 less intensive reclamation approaches. Habitat for biodiversity function was
613 similar in the three treatments, but low due to the dominance of introduced species
614 in the mesocosms. Future reclamation actions should combine modification of soil
615 conditions and improvement of the starting plant community (*i.e.* seeding or
616 planting of native ultramafic species).

617

618 **Conclusion**

619 In this work, we have assessed the impact of three reclamation techniques for post-
620 operation ultramafic sites (*i.e.* topsoil application, non-compacted, and organic
621 amendment) on the level of four ecosystem functions. These four key ecosystem
622 functions for ultramafic areas were chosen according to our literature review:
623 biomass production, nutrient cycling, water filtration, and habitat for plant
624 biodiversity. A large set of measures on soil chemistry, microbial activity, plant
625 and water were measured to assess those ecosystem functions and an evaluation of

626 global ecosystem services would have mean a larger measurement effort (*e.g.* soil
627 physics, fauna). In order to process this large dataset of multiple variables we have
628 suggested a first approach for a multicriteria analysis. Such an approach would be
629 useful for the assessment of ecosystem services. However, the sensitivity of the
630 ranking and the computation have to be tested to assess the robustness of this
631 approach.

632 In the short-term of our study (one year), the three reclamation techniques all
633 improved the level of the ecosystem functions in comparison with literature data
634 on degraded sites.

635 The reclamation effort on a given site must balance the recovery targets, the
636 economic costs and the energy consumption. However, our results have shown that
637 increasing reclamation effort can be beneficial, detrimental or even have no effect,
638 depending on the considered parameter and ecosystem function. Longer studies
639 (5–10 years) are also needed to assess the evolution of the system in the mid and
640 long term. The recommendation of one reclamation approach over the other is not
641 straightforward, in the light of contrasting effects of our treatments. However,
642 generated information will be useful to selected choice the strategy for the
643 recovery of degraded tropical serpentinite quarries and dump sites.

644

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655

656

657 5. References

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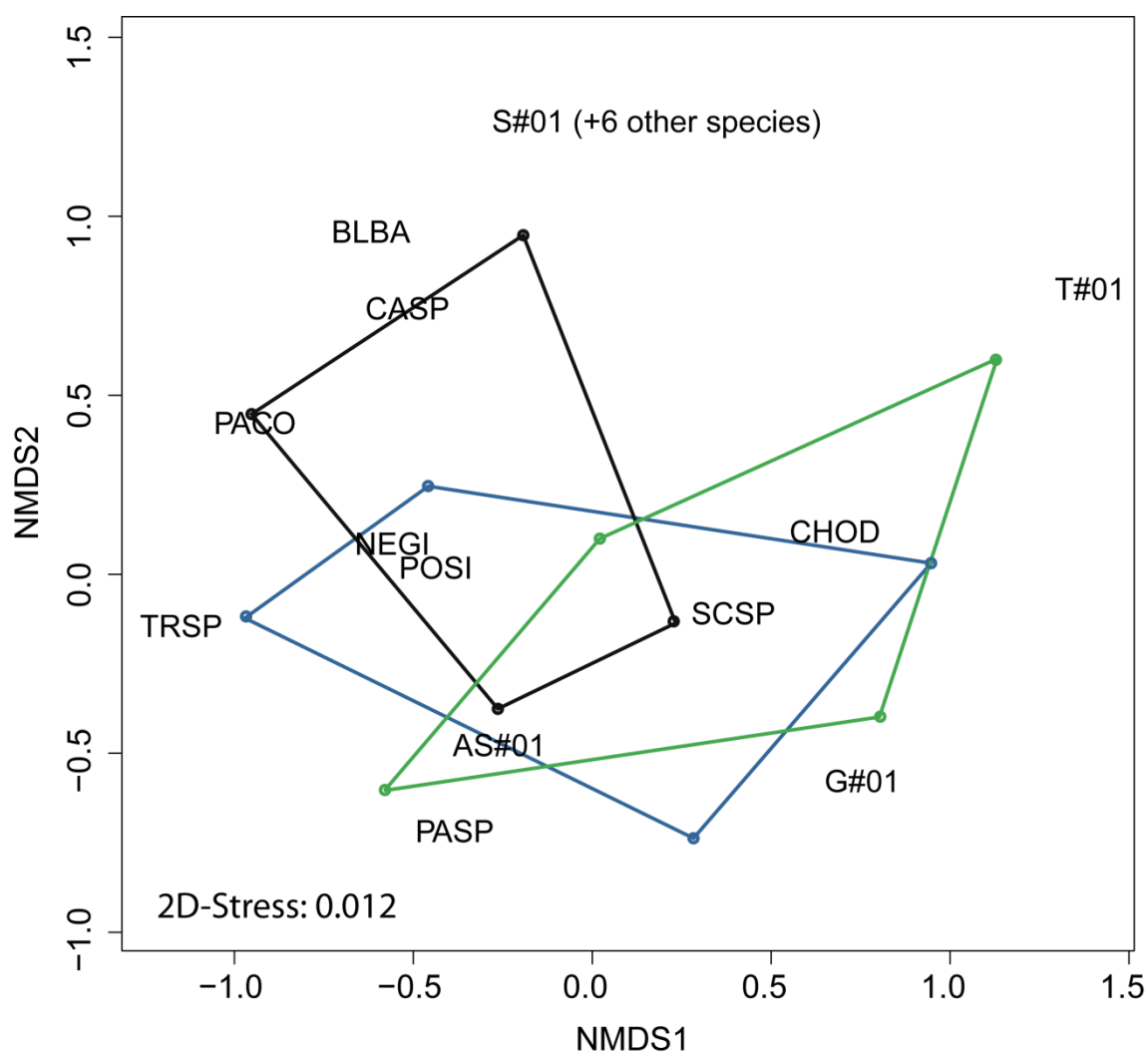
SUPPLEMENTARY MATERIAL

Title: Effects of increasing reclamation effort on ecosystem functions of human-degraded soils from ultramafic areas

Authors: C. Quintela-Sabaris et al.

Journal: Journal of Geochemical Exploration

Figure S1: NMDS analysis of plant communities growing on mesocosms. Dots mark the position of the 12 mesocosms. Coloured polygons are convex hulls connecting pots with same treatment: blue (COM), green (NC) and black (OM). Position of plant species are indicated by four-character codes (see table 2).



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