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The Ni biogeochemical cycle in a tropical agromining crop system

Romane Tisserand

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Thesis

presented for the degree of

Doctor of the University of Lorraine

Specialisation **Agronomic Sciences**

by

Romane Tisserand

The Ni biogeochemical cycle in a tropical agromining crop system

Publicly defended on 12th February 2021

In front of the Jury composed by :

Aïda BANI	Professor, University of Tirana, Albania	Rewiever 1
Markus PUSCHENREITER	Professor, University of Natural Resources and Life Sciences Vienna, Austria	Rewiever 2
Joseph POLLARD	Professor, Furman University, USA	Examiner 1
Marie-Odile SIMONNOT	Professor, Université de Lorraine, France	Examiner 2
Emmanuelle MONTARGÈS- PELLETIER	Research officer, CNRS, France	Guest 1
Bernhard Zeller	Senior Scientist, INRA, France	Guest 2
Guillaume ECHEVARRIA	Professor, Université de Lorraine, France	Thesis Director
Antony VAN DER ENT	Senior Research Fellow, University of Queensland, Australia	Codirector

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List of abbreviations

Al	Aluminium (element)
Ca	Calcium (element)
Co	Cobalt (element)
Cr	Chromium (element)
Cu	Copper (element)
CuO	Copper oxide (compound)
DBH	Diameter breast height
DMG	dimethylglyoxime
Fe	Iron (element)
Ha	Hectare (unit)
H ₂ O ₂	Hydrogen peroxide (compound)
HNO ₃	Nitric acid (compound)
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
K	Potassium (element)
LOD	Limit of detection
Mg	Magnesium (element)
MgO	Magnesium oxide (compound)
Mg:Ca	Magnesium to calcium quotient
Mn	Manganese (element)
MnO ₂	Manganese oxide (compound)
Na	Sodium (element)
Ni	Nickel (element)
NiO	Nickel oxide (compound)
P	Phosphorus (element)
pH	(soil) acidity
r^2	squared regression coefficient
S	Sulfur (element)
µm	Micro-metre (unit)
XRF	X-ray Fluorescence
Wt	Dry
Zn	Zinc (element)

Scientific development

☼ Three manuscripts as first author in submission or on publication:

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Tisserand R, Nkrumah PN, Van der ent A, Zeller B, Sumail S and G Echeverria (submitted in November 2020) Biogeochemical cycling of nickel in a high-density stand of the hyperaccumulator *Phyllanthus rufuschaneyi* in Sabah, Malaysia.

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☼ Two manuscripts as second author:

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☼ Posters and oral communications

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General INTRODUCTION

Ultramafic rocks are composed mainly of mafic minerals and consist of ferromagnesian minerals subducted at continental margins; this type of rock cover more than 3% of the Earth's surface (Brooks, 1987; Guillot and Hattori 2013). Ultramafic regions are areas of interest for the establishment of Ni mines, such as in New Caledonia or in Indonesia. Open cast mines of lateritic nickel ore have a negative impact on the biodiversity of these areas, notably by the destruction of the soil and plant cover. The flora of these regions is very particular due to the specific edaphic environment, with toxic concentrations of Mg and Ni, and a dearth of mineral nutrients (Proctor et al. 1999). Studying plant adaptation to ultramafic soils is valuable for evolutionary ecologists and geneticists (Brady et al. 2005).

Indeed, some plants restricted to ultramafic soils have evolved ecophysiological mechanisms to accumulate trace elements to extreme concentrations; they are known as hyperaccumulator plants (Reeves 2003; van der Ent et al. 2013). In Sabah, 31 plant species are currently known as Ni-hyperaccumulators, which occur mainly on Hypermagnesian Cambisols (van der Ent et al. 2016a, b, 2019). Malpighiales order is specially attractive for research of new taxa of interest because of the discovery of so many several trace element hyperaccumulator plant species in this order that may have applications in phytomining (Reeves 2003). A large number of Ni hyperaccumulator plants belong to this order (Malpighiales), which in turn, belongs to the COM clade (Jaffré et al. 2013; Reeves et al. 2017). This order also contains the family with the greatest number of Ni hyperaccumulator species, *i.e.* the Phyllanthaceae Family in Cuba and New Caledonia (Reeves 2003; Jaffré et al. 2013).

In the recent decades, global demand for strategic metals has increased and it will continue to increase, notably for Ni. New methods of metal extraction emerged that could challenge traditional mining techniques. Agromining is one of them, and it is a chain of processes where metal hyperaccumulator plants are cultivated and harvested for the recovery metal bio-ores. The objective of this technology is to produce metals at a relatively low environmental cost and possibly at a low economic cost, enough to compete with traditional mining techniques. This cultivation method can be developed on soils that are naturally enriched in metals, such as ultramafic soils or on mines wastes from which the extraction by conventional metallurgical methods is not economically viable. This process is currently studied under temperate regions with very promising results. For example, a Ni yield of $145 \text{ kg ha}^{-1} \text{ y}^{-1}$, can be expected in Albania using the Ni hyperaccumulator *Alyssum murale* (Bani and Echevarria 2019). In the Tropics, notably in Malaysia, substantial unrealised opportunities exist for economic Ni Agromining (Nkrumah et al. 2018).

The study of the dynamics of the Ni pools in ultramafic soils and their consequent quantification are crucial to determine the potential duration and viability of Ni agromining. The pools of available Ni in soils will be strongly depleted by the continuous uptake by hyperaccumulators and their subsequent harvest. Recent studies have demonstrated that hyperaccumulator plants directly influence the composition of available and total Ni pools in the top horizons of ultramafic soils (Estrade et al. 2015). Ni hyperaccumulator plants could play a major role in maintaining high concentrations of Ni in the surface of soils during weathering and pedogenesis (Echevarria 2018).

In order to be suitable crops for agromining, hyperaccumulators need to accumulate Ni at elevated concentrations, such as “hypernickelophores” that accumulate Ni at concentrations above 1% wt. Such crops need to be easily to cultivate, to grow reasonably fast and, to produce a large amount of biomass. *Phyllanthus rufuschaneyi*, a newly-described species, is known to be restricted to only two localities in Sabah (*i.e.* Bukit Hampuan and Lompoyou Hill) making this plant extremely rare (Bouman et al. 2018). The soil type in its native habitat is a young (eroded) hypermagnesian Cambisols on strongly serpentinised bedrock (van der Ent et al. 2018). This plant accumulates exceptional Ni concentrations in its shoots, with up to 2.5 wt% in old leaves and 1.2 wt% in twigs (van der Ent *et al.* 2015; van der Ent and Mulligan 2015; Bouman et al. 2018). The Ni concentrations in the phloem sap and in the seeds are also very high, with mean concentrations of 0.9 wt% and 1.8 wt% (van der Ent and Mulligan 2015). Considering the high shoot Ni concentrations, coupled with its multi-stemmed habit, rapid regrowth after coppicing, light-demanding, and pest resistance, *P. rufuschaneyi* is an ideal ‘metal crop’ candidate for tropical Ni agromining (Bouman et al. 2018; Nkrumah et al. 2019a). Therefore, this tropical hypernickelophore species was selected to be deployed in field full-scale experiments as the best candidate in the conditions of Sabah (Nkrumah et al. 2019d), and was chosen as a candidate species for this PhD research to assess the impact of hyperaccumulation in the biogeochemical cycling of Ni in ultramafic ecosystems.

Nevertheless, the factors and treatments to increase Ni production previously studied under temperate conditions (Bani et al. 2015; Nkrumah et al. 2016), may not be the same in the tropics due to the specific characteristics of tropical hyperaccumulator plants. The agronomy of tropical ‘metal crops’ had not been tested until Nkrumah et al. (2018d) investigated the conditions to optimise the growth and the development of two hypernickelophores of Sabah (Malaysia). The response of *Rinorea* aff. *bengalensis* and *Phyllanthus rufuschaneyi* to the amendment of different elements was studied through experiments in pots (Nkrumah et al. 2019b,c,d). The Ni yield of *P. rufuschaneyi* decreases with N and OM input, contrary response to Ca and S addition. Nevertheless, the Ni yield of this tropical

hypernickelophore could potentially reach $250 \text{ kg ha}^{-1} \text{ y}^{-1}$ (Nkrumah et al. 2019a). Field trials under tropical conditions will help to improve the performance of any potential agromining crop. Agromining of Ni could have high economic potential, but large-scale demonstrations are surely needed to provide convincing evidence for commercial operations (Reeves et al. 2018b; Nkrumah et al. 2019a).

Biogeochemical cycles of tropical hyperaccumulator plants are still poorly understood. Describing the dynamics of Ni within the soil-hyperaccumulator plants ecosystem is necessary to elucidate the ecological role of hyperaccumulator plants in their natural environment, but also to understand their potential behaviour under tropical agromining systems. Litterfall and decay seem to affect Ni return to the soil, the study of both internal and external Ni cycles are needed to explicitly understand the importance of hyperaccumulator biomass and Ni cycling. Agromining and exporting Ni rich-biomass will interrupt the cycle. Hyperaccumulator plants are also capable of exporting high contents of nutrients from soils (e.g. Ca K P and S). Therefore, it is important to assess the impact of soil fertility in long term field conditions on the cycle of these elements and on the agronomy of these plants.

To summarise, the key questions that this PhD will address are the following:

- ⊗ What are the Ni cycles (internal and external) and their impact on the ecological functioning of tropical hyperaccumulator forest? What is the role of hyperaccumulator plants in the ecological succession on ultramafic soil?
- ⊗ How rapid are the Ni fluxes across the soil-plant compartments and what is the turnover of Ni in a hyperaccumulator tropical system? How fast can a tropical Agromining crop can deplete Ni in soil?
- ⊗ What are the impacts of inorganic and organic fertilisation on the sustainability of a tropical Ni agromining crop?

The main objective of this PhD is the characterisation of metal and nutrient cycles of tropical hyperaccumulator plants in both a planted agromining field plot and in a natural stand (secondary forest). Moreover, many additional fundamental questions arise, including the role of hyperaccumulation in ultramafic pedogenesis, and the efficiency of nutrient and metal biogeochemical cycling in hyperaccumulator plant stands.

From these scientific questions, the following objectives arise:

- ❁ To study the biogeochemical cycling of a natural forest of *P. rufuschaneyi* in order to assess and evaluate the natural fluxes of Ni in the ecosystem;
- ❁ To manipulate such an ecosystem in order to perform a sensitivity test of the ecosystem for the following flux: litter return to the soil; from no return (export) to a doubling of the return.
- ❁ Based on both the understanding of the role of the litter/Ni biogeochemical cycling in feeding *P. rufuschaneyi* and the plant NPK and Ni requirements, to optimize the cropping system of *P. rufuschaneyi* for Ni agromining.

This thesis is structured in four chapters in order to address the three objectives. The first chapter corresponds to the state of the art, which presents the most recent stage of the research in tropical Agromining. The biogeochemical behaviour of natural hypernickelophore forest is described in the chapter two, while the behaviour of a tropical Agromining farm is presented in chapter three. Chapter four is devoted to tropical Agromining improvement by organic and chemical fertilisation.

CHAPTER 1: State of the Art

A. Ultramafic soils

Soil in general

Soil is the surface layer of the earth's crust at the interface between the lithosphere, biosphere, atmosphere and hydrosphere (Sumner 2000). The soil is a heterogeneous medium composed of interacting gaseous, liquid and solid phases. The solid phase is composed of a mineral and organic part. The latter comes from both plant and animal residues. The mineral part can come from the degradation of the mother rock, from any other natural or from unnatural mineral input. The composition of the mother rock will influence the composition of the soil. It is possible to find unaltered minerals from the mother rock in the soil; they are called primary minerals. Other minerals are altered by chemical and physical modifications, these secondary minerals are more or less well crystallised. The latter tend to be more reactive than unweathered minerals. Among them, oxides and clays are groups of highly reactive minerals that influence, among other things, the capacity of soils to exchange nutrients and metals. Plants take elements from the water solution or from the exchangeable fraction.

Chemical elements in soils are commonly categorised as major (O, Si, Al, Fe, Ca, Na, K, Mg and P) and minor elements (Cu, Co, Zn, Cr, Ni, Cd...). Minor elements, also called trace elements, are negligible chemical constituents of soils due to their low concentrations but may be essential as micronutrients for plants (Kabata-Pendias and Pendias 2001).

Ultramafic non-anthropised soils

Ultramafic soils represent 1% of the surface area and are present at the former tectonic margins since their ultrabasic parent rock comes from fragments of the oceanic lithosphere (Brooks 1987; Coleman and Jove 1991);. This type of soil occurs in all the continents, in different countries such as Albania, Greece, Austria, Spain, Indonesia, Malaysia... These rocks are very poor in silica (<45%) and contain more than 90% of mafic minerals (*i.e.* rich in Iron and Magnesium) (Le Bas and Streckeisen, 1991). This bedrock contains very limited contents of major nutrients, which induces a low nutrient concentration in derived ultramafic soils, including Ca, P and K. These soils are also characterised by significant concentrations of metals, like Ni, Co and Cr. Their formation is unique and they cannot be compared to adjacent soils evolving under the same climatic conditions but on other types of bedrock. Nickel quantity and speciation also depend of the position in the toposequence (Cheng et al. 2011). There are different types of ultramafic soils which are classified according to the weathering of their parent rock:

- ⊗ Ultramafic Leptosols or Regosols: young, shallow and therefore not very altered soils with many primary minerals. Their pH is high, and their CEC is dominated by Mg.
- ⊗ Ultramafic Cambisols: moderately deep and weathered soils, with both primary and secondary minerals. Their pH is neutral or slightly acidic.
- ⊗ Ultramafic Ferralsols: old, very deep soils with many secondary minerals due to the extensive weathering they have undergone. Ni is concentrated at more than 1% in secondary oxides, smectites and hydrated magnesium silicates.

Ultramafic soils contain 1400 to 2000 mg kg⁻¹ of Ni (Kabata-Pendias et Pendias 2001). In Brazil, values of up to 27,000 mg kg⁻¹ total Ni in soils have been measured (Becquer et al. 2005). In these highly weathered soils, Ni is mainly related to secondary minerals. In Southeast Asia, ultramafic soils are relatively abundant, particularly in Indonesia and Malaysia, with a known surface area of about 23,000 km² (van der Ent et al. 2013; Galey et al. 2017). In Malaysia, in the state of Sabah, the ultramafic land area is estimated at 3500 km² (Proctor et al. 1988; Repin 1998). In this region most soils derived from serpentine rocks are Cambisols, which induce a significant potential Ni bioavailability (van der Ent et al. 2018).

Ultramafic anthropised soils

Nickel in soils can come both from the parent rock and from anthropogenic deposits or inputs (Kabata-Pendias, 1993). In South Asia and the Pacific, there are numerous mining operations, including open-pit Ni mines. These mines are usually found in New Caledonia (France), Sulawesi (Indonesia) and Palawan (Philippines). Ferralsols also contain economically exploitable quantities of Ni and Co (e.g. Wilburn 2012). Declining Ni sulphide reserves have led to increased exploitation of Ni from laterites (Taylor 1995; Mudd 2009). Currently, more than 60% of the Ni produced in the world comes from laterites. The mineralogy and purity of the deposit vary according to its formation, which is often various. The most concentrated deposits are found in areas where the leached Ni is concentrated in the neoformed silicates of the saprolite and where oxide-rich regoliths are close to the surface. Some Ferralsols may contain more than 3% of Ni and can be potentially used for commercial agromining.

Nickel availability

The transfer capacity of an element from the soil to the roots of the plants is entitled “phytoavailability” (Barber 1995). Bioavailability can be measured with DTPA (diethylenetriaminepentaacetic acid) extraction. Nevertheless, this extraction can solubilise more element than expected and because each plant has a different behaviour, especially hyperaccumulators plants. The term ultramafic does not give information on the behaviour of metals and its availability to plants (Nkrumah et al. 2019d). The bioavailability of Ni varies according to many factors. The pH seems to be the factor that most influences the solubility of Ni in soils (Echevarria et al. 2006). The oxidation-reduction potential must also be taken into account, especially when studying ultramafic soils, as a low value favours the solubility of the hydroxides. The soil temperature and soil moisture may not be neglected as they affect soil processes. Indeed, Ni availability can decrease with increasing soil moisture (Angle 2003). Nickel can be bound to three minerals in general: oxides, clays and hydrated magnesium silicates. The form of the metal influences its bioavailability to the plant. Depending on the soil processes involved, clay minerals such as smectite or poorly crystallised iron oxides are the most important carrier phases for the available Ni (Massoura et al. 2006). Nickel bound to crystallised iron oxides is very little available. Nickel availability is higher in moderately weathered soils than in highly weathered soils where the main mineral is goethite. The bioavailability of Ni in anthropised soils is more challenging to characterise and to explain. Soil organic matter can also trap metals (Kabata-Pendias and Pendias 2001). The effect of organic matter on the bioavailability of metals is difficult to predict. The origin and type of organic matter influence the mobility of Ni.

B. Biogeochemical cycles in ultramafic soils

a. Biogeochemical cycles in general

According to Switzer and Nelson (1972), biogeochemical cycles consist of three sub-cycles (Figure 1):

- ♻️ The geochemical sub-cycle: in an agrosystem, a part of the nutrients is exported out of the plot because of crop harvesting and leaching with draining water. Nutrients can be added to the soil through atmospheric deposition, weathering of the bedrock or anthropogenic inputs (*e.g.* when organic and/or inorganic matter is amended).

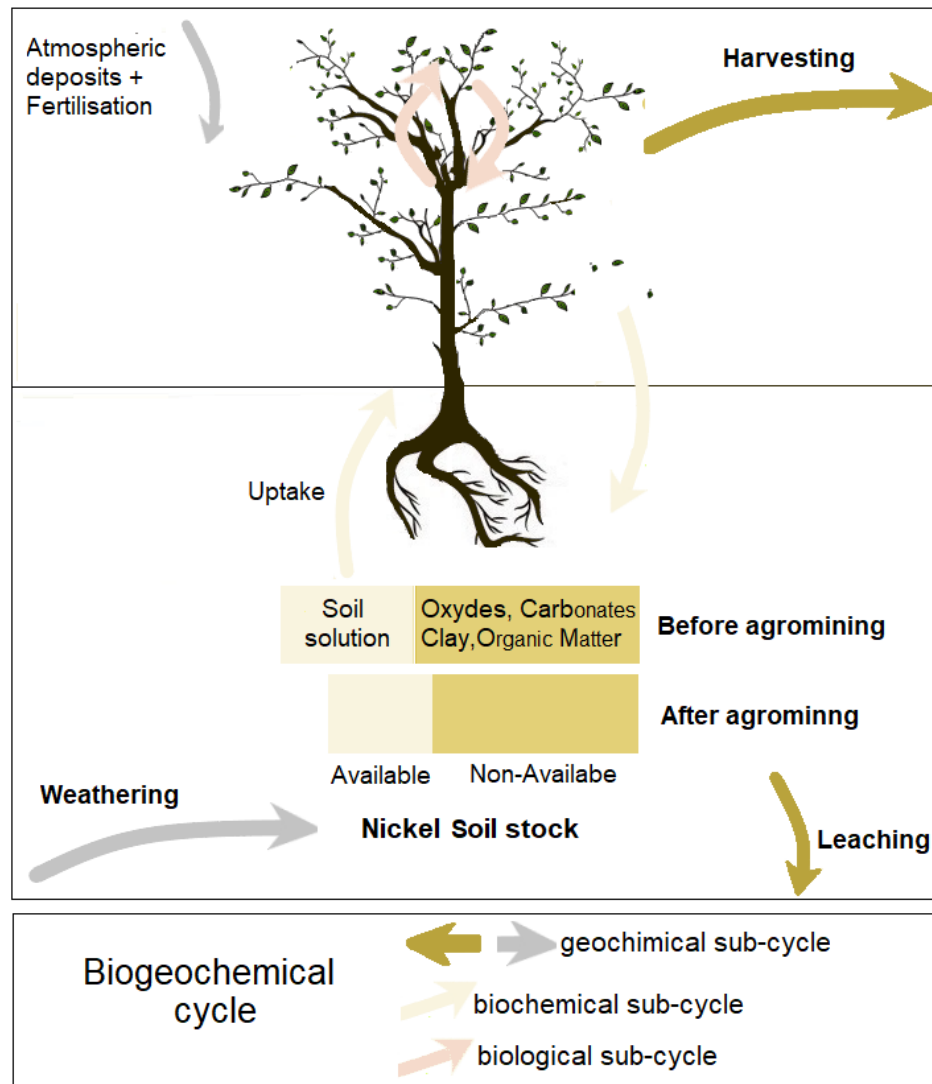


Figure 1 Biogeochemical cycles under harvesting

- 🌀 The biochemical sub-cycle: the plant itself can recover the elements that it returned to the soil after litterfall and decay. Elements from litter degradation can be taken up by roots.
- 🌀 The biological sub-cycle: there is an internal cycle within the plant. The elements can be transferred to the different organs of the plant itself. For example, before leaf senescence, plants can remobilise elements in their other organs.

Elements output-input budget are essential to know because it is an indicator of soil fertility and management sustainability. It can be used for practical recommendations, such as intensity of crop harvesting and fertiliser input optimisation (Ranger and Turpault 1999). Unbalanced budget indicates a depletion in elements of the ecosystem. However, a balanced budget does not indicate any change in the

ecosystem. The budget can calculate in forests plantations as follow (Ranger and Turpault 1999): input (atmospheric deposits+weathering)– output (harvesting+drainage). Other fluxes have to be taken into account if they are not negligible, as throughfall, litterfall, anthropogenic inputs...

Litterfall of numerous tropical humid forests is important compared to other forests (e.g. Bray and Gorham 1964;). In Borneo forest, litterfall of 11 t ha^{-1} , so of 1.1 kg m^{-2} , have been recorded (Burghouts et al. 1992, 1998). A study in 23 tropical forests reported a decomposition of the litter mass of more than 95% mass within a year in most of these sites (Powers et al. 2009). Litterfall is more pronounced during dry season in comparison with rainy season (Nakagawa et al. 2019) but this observation is not always the case in a wet tropical forest (e.g. Saner et al. 2012). In a dipterocarp forest in Sabah, litterfall and litter decomposition were not found to be associated with the rainfall pattern (Burghouts et al. 1992).

Carbon cycle

In soils, there are two types of carbon, inorganic and organic carbon. The first one comes from the weathering of the bedrock or from atmospheric deposits. Living organisms produce the second type. One of the main stocks of carbon is sedimentary rocks. Plants capture atmospheric CO_2 during photosynthesis by using solar energy to synthesise organic matter and fix carbon in carbohydrates. This captured carbon is either returned through respiration to the atmosphere or retained by the plant. A minor fraction can also be drained from the soil under the bicarbonate form. The carbon stock in the soil is positively influenced by the input of plant and animal debris but negatively by their decomposition, which implies respiration. All types of soils cannot store the same amounts of carbon because of their nature (pH, carbonate content,...) and also because of their use (forest, grassland, tillage, no till,...).

Principal nutrients cycles

Even within a same climate zone, the intensity of each of the fluxes vary strongly among types of ecosystems. In tropical rain forests, nutrients are most of the time strongly incorporated in plant biomass and the forest floor (Vogt et al. 1986). Litterfall represents the main pathway of nutrient transfer from the vegetation to the soil; most nutrients absorbed by plants return to the soil as leaf litter and sometimes as branches and logs (Vitousek 1982, Attiwill and Adams 1993). Potassium appears to be the most re-absorbed element before senescence of leaves and is mostly transferred by throughfall (Vitousek and Sanford 1986). Whereas, Ca is one of the less reabsorbed element (Vergutz 2012). Phosphorus is an

essential element for the development of the flora despite its low accumulation in plants. The behaviour of P in tropical forests diverges from the temperate forest, because P availability constrains the productivity of most lowland tropical forests as one of the main limiting factors (Vitousek 1984).

Nickel cycle

Ultramafic outcrops are the most important sources of Ni in the terrestrial ecosystems (Estrade et al. 2015). Few studies focus on hyperaccumulator litter degradation and its potential effect on Ni recycling. Echevarria (2018) studied *Odontarrhena chalcidica* plants and found that almost half of the Ni contained in leaves after decaying was leached away after only a few months. Boyd et al. (2008) showed that the degradation of herbaceous hyperaccumulators can be fast. Nickel in decomposing hyperaccumulator leaves was released rapidly: after one month 57–68% of biomass was lost, and only 9–28% of original Ni content remained. The litter of Ni hyperaccumulator species has rapid decomposition rates compared to that of non-hyperaccumulator plants (Adamidis et al. 2016). Furthermore, Ni addition to litter of tropical trees added no significant effect on its degradation (Powers and Salute 2011). Large quantities of lighter Ni isotope having biotic origin Ni are found in the upper soil layer of hyperaccumulator forest (Zelano et al. 2020).

Metal and nutrients uptake modeling

Different model exists to predict Ni availability and uptake by plants.

Pedotransference functions can be used to predict specific soil properties using data from soil surveys (Bouma 1989). Chemical analyses can be carried out to parametrise the plant model on vegetation stock. The bioavailability of the target element needs to be simplified to predict elements behaviour. For most metals, the main parameters to be taken into account seems to be the soil pH, the nature and properties of the sorbent phases, the cation exchange capacity (CEC) and the redox potential (Eh).

Model can be based on the mechanistic approach as Barber-Cushman model that is used to model major element uptake by plants (Barber 1995). It have been used to characterized hyperaccumulator plants (e.g. Sterckeman et al. 2004; Puschenreiter et al. 2005) but it do not take into account all the ecophysiological functioning of the plant.

C. Ultramafic soil flora

The chemical composition of ultramafic soils makes them areas of high biodiversity. In various ultramafic regions of the world, there is a high level of endemism because of the geochemical peculiarities of these regions. In Balkans, biodiversity and endemism of plants are high on serpentines (335 taxa) (Stevanović et al. 2003). The state of Sabah (Malaysia) is one of the top five areas with the highest density of species diversity at global scale (Barthlott et al. 2017).

a. Metal tolerance mechanisms

Roots absorb metals from the soil solution through passive transport (diffusion) along a gradient of concentration or active transport against concentration gradient (Campbell et al. 2012). For Ni in solution, the form absorbed by roots is the free ion Ni^{2+} . Plants react differently and according to the metals they encounter. In general, Ni is absorbed passively while Zn is absorbed actively by plant (Kabata-Pendias and Pendias 2001). After uptake, Ni can be complexed by chelating agent inside the root cells or the cell vacuoles, as oxalate or malate acid (Yang et al. 2005; Nascimento and Xing 2006). This transformation turns the element less toxic for the plant because it does not impair its physiology. Metals are generally stored in vacuoles because they are less responsive to oxidation (Eapen and D'souza 2005; Yang et al. 2005). Nickel stock varies in function of the plants studied, it is often found on the cell wall (Yang et al. 2005). Some metals, such as Fe, Zn, Cu, and Ni, act mainly as co-factors for enzyme activity. For instance, Ni is required by plants for the activity of urease, an enzyme which is involved in the N-NH₄ cycle in plants (Polacco et al. 2013) and its concentration is low and generally ranges between 0.01 and 5 mg kg⁻¹ dry matter (Adriano 2001).

Plants can be “sensitive” or “tolerant” to high metal concentrations in soils. Sensitive plants can exhibit symptoms of toxicity such as slow/reduced plant growth or leaf chlorosis. Metal tolerance implies an adaptation which results in a change in the physiological response of the plant. These mechanisms are metal (i) exclusion, (ii) indication or (iii) (hyper)accumulation. Many plants exclude one or more metals; these plants reduce root metal uptake and translocation to the aerial parts and are common on ultramafic soils (Gonnelli and Renella 2013). Indicator plants accumulate a number of elements reflecting the metal concentrations in their substrates and often show no specific regulation of metal uptake neither at the level of root uptake, nor at the level of xylem uploading. Other plants can accumulate one or more metals without exceeding the hyperaccumulation thresholds. Some relatively rare plants can accumulate abnormal concentrations (*i.e.* more than 100 to 1000 times more than non-accumulating species on the

same soil) of one or more metals in their aerial biomass, they are called hyperaccumulators (van der Ent et al. 2013).

The hyperaccumulation limits vary according to each metal, the limit is 300 mg kg^{-1} for cobalt, 1000 mg kg^{-1} for Ni, 3000 mg kg^{-1} for Zn and $10\,000 \text{ mg kg}^{-1}$ for Mn (van der Ent et al. 2013). Currently, more than 500 species of plants are known to accumulate more than 0.1% wt of Ni (Reeves et al. 2018) whose almost 300 in tropical regions (Reeves et al. 2018, Gei et al. 2020, Navarette et al. 2021) (Figure 2). For plants hyperaccumulating Ni, an additional threshold has been defined; a plant accumulating more than 1% wt of Ni is considered a hypernickelophore (Jaffré and Schmidt 1974). Sap can be strongly enriched in Ni, up to more than 20% as for *Pycnanandra* (syn. *Sebertia*) *acuminata* (Jaffré et al. 1976). The capacity of these plants to accumulate metals has evolved in relation to the high bioavailability of metals, particularly Ni and Co, in ultramafic environments.



Figure 2 Ni hyperaccumulator hotspots in tropical regions (Reeves et al. 2018, Gei et al. 2020, Navarette et al. 2021)

Hyperaccumulation purpose

Hyperaccumulator plants are almost exclusively found in Angiosperms, belonging to more than 50 families (Krämer 2010; Reeves et al. 2018). Among them, the Brassicaceae, Euphorbiaceae, Phyllanthaceae, Salicaceae and Violaceae are the most representative families with the highest number of Ni hyperaccumulator species (Krämer 2010; Reeves et al. 2018; Bouman et al. 2018). Currently, there is a scarcity of studies on the molecular mechanisms of Ni hyperaccumulation. Ni hyperaccumulation seems to imply the expression of diverse transporters at different crucial steps, iron transporters being among the most important (Merlot et al. 2018). Hyperaccumulation is a stress resistance trait that appeared multiple times and on different plant species during evolution and has persisted in several groups of plants (e.g. families) for which significantly higher proportions of hyperaccumulators than in other groups have been recorded (Jaffré et al. 2013). Hyperaccumulation should confer some advantages for the survival of these species. Nevertheless, this

advantage is still not well understood, and some hypothesis have been proposed over the years (Tadros 1957; Boyd and Martens 1992; Pollard et al. 2014) :

- ☼ Mechanism of metal tolerance
- ☼ Mechanism of drought resistance
- ☼ Mechanism of allelopathic interference with competing plants
- ☼ Consequence of efficient nutrient uptake
- ☼ Defence against herbivores or/and pathogens

Some hypothesis are more plausible than others. The “defence against herbivores or/and pathogens” obtained the most supporting evidence (Boyd 2004, 2007) but it does not reach a consensus (*i.e.* Noret 2007). Multiple theories could explain at the same time plant tolerance and accumulation. Links between biotic stresses and metal homeostasis in metal hyperaccumulators are easily descriptive and could be useful in further studies (Kramer. 2010)

Nevertheless, some hyperaccumulators are only found on ultramafic soils whilst some other hyperaccumulators grow well on both ultramafic and non-ultramafic soils. The first are called obligate hyperaccumulator and the second are called facultative hyperaccumulators. The latter would have evaluated from obligate hyperaccumulator in purpose of expanding their range to include non-metalliferous soils (Pollard et al. 2014). New hyperaccumulator plant species continue to be discovered whether it is in temperate or tropical regions (Nkrumah et al. 2018; McCartha et al. 2019; van der Ent et



Figure 3 *Phyllanthus rufuschaneyi* in a forest (left) and *Rinorea bengalensis* in pots (right)

al. 2019).

Hyperaccumulator plants

In the Mediterranean region, Ni hyperaccumulation is mainly found in the Brassicaceae family, *i.e.* in the *Odontarrhena*, *Bornmuellera*, *Arabidopsis* and *Noccaea* genera. The first Ni hyperaccumulator was discovered in 1948; its name was then *Alyssum bertolonii* (Minguzzi and Vergnano 1948), now *Odontarrhena bertolonii*. Pioneer studies were majority setting up with plants of the genus *Odontarrhena* (syn. *Alyssum*) (Li et al. 2003 and Bani 2007).

Numerous Ni hyperaccumulators, and notably hypernickelophores, occur in Southeast-Asia, such as *Xylosma luzoniensis*, *Antidesma montis-silam*, *Dichapetalum gelonioides* or *Psychotria sarmentosa* which occur in Sabah (Nkrumah et al. 2018, 2019; van der Ent et al. 2018, 2019). Currently, 48 plants in Malaysia and 18 in Halmahera are known to accumulate more than 0.1 wt. % of Ni (van der Ent et al. 2018, 2019; Lopez et al. 2019). Tropical hyperaccumulator plants are mostly perennial shrubs or trees that could potentially regenerate above their ground biomass rapidly after harvesting. The most interesting species in Malaysia are from Phyllanthaceae and Violaceae family (Figure 3). The *Phyllanthus* genus contains many Ni hyperaccumulator endemic species in several regions of the world (Cuba, New Caledonia, Borneo and Palawan islands), suggesting an active and continuous evolving speciation towards hyperaccumulation. *Phyllanthus rufuschaneyi* and *Rinorea cf. bengalensis* are two hypernickelophores endemic from Sabah with high biomass production (Figure 4) (Nkrumah et al. 2016a, 2018d; van der Ent et al. 2015a). In these two plants, Ni is associated with citrate and stored in the epidermis and in the spongy mesophyll (Van der ent et al. 2017). *Rinorea cf. bengalensis* has the peculiarity of displaying Ni-rich phloem tissues with a stunning jade-green colour due to the high concentration of Ni ions (Van der ent et al. 2017).

D. Phytoremediation: soil rehabilitation techniques using plants

The vast capacity of metal hyperaccumulator plants to respond to the presence of high metal concentrations in soils makes them of great interest for soil remediation applications (Baker et al. 1994). Since the early eighties, many processes of cleaning up metal contaminated soil with hyperaccumulator plants are studied (Chaney 1983). Phytoremediation is a technology that uses tolerant plants to clean up soil, water or air contaminated by pollutants (Salt et al. 1998). It brings together different techniques that can be complementary

- ☼ Phytoextraction uses accumulating or hyperaccumulating plants in order to harvest metals from the aerial parts of plant.

- ☼ Phytodegradation uses the associations between plants and microorganisms in order to degrade the pollutants.
- ☼ Phytostabilisation uses plants to immobilise pollutants in the soil to prevent their migration and dissemination in other compartments of the environment.
- ☼ Phytovolatilization makes it possible to transform polluting compounds from the soil into volatile compounds.
- ☼ Rhizofiltration allows the roots to absorb and accumulate pollutants from the waste to purify them acting as a filter.

Phytoextraction is an inexpensive technique that allows to maintain or even improve the biological quality of the original soil. It is an alternative to traditional metal extraction techniques such as heat or acid treatments. Phytomining was first described in the early 80s as the use of phytoextraction technologies using hyperaccumulators to produce valuable metals from soils (Chaney 1983). First phytomining trials were realised in the 90's (*i.e.* Nicks and Chambers 1998). This technology displays numerous advantages such as low cost, respect for the environment and good acceptance by the public. Nevertheless, this technique requires long periods of time, from some years to several decades depending on the metal considered, the contamination rate and its availability in the soil.

E. Agromining : How to produce metals with phytoremediation?

Agromining is a recent concept which phytomining is the first stage and metallurgical processes are the second stage (Van der Ent, 2015). Agromining is, therefore, a chain of processes that cover at the same time the improvement of the soil quality, the cropping system, the incineration of the biomass produced and the metal recovery from the ashes of the hyperaccumulator plants which can be considered as a bio-ore (van der Ent, 2015). As for phytomining, its viability depends on the world price of other metals. Nickel agromining is considered an economically viable technology (Chaney et al. 2018; Kidd et al. 2018; Nkrumah et al. 2019).

a. Tropical model for plants and soils

To be used in Agromining, hyperaccumulators plants must possess certain traits, such as high biomass yield combined with high Ni concentrations in the above-ground biomass (more than 1%) (Chaney et al. 2007). Currently, 16 species have been identified in tropics as having exceptionally high potential for use

as ‘metal crops’ for Ni agromining (Nkrumah et al. 2020). Local plant species are always recommended due to their adaptation to climate conditions and to avoid the introduction of potentially invasive species (Baker 1999; Bani et al. 2007).

Using the term ‘ultramafic’ alone is not sufficient to identify potentially Ni agromining sites due to their variable Ni availabilities (Nkrumah et al. 2019a). Ultramafic Cambisols from North Borneo are characterised by circum-neutral pH and high DTPA-extractable Ni, (above 200 ppm of Ni). They have a high potential Ni uptake by plants, and therefore, a high potential for Ni agromining (van der Ent et al. 2017, 2018; Echevarria, 2018). Young shallow ultramafic soils, named Leptosol and also old Ferrasol occurring in Southeast Asia seem to have a low potential for tropical agromining due to their lower Ni availability. However, the mix of these substrates with ultramafic cambisols seems to increase Ni yields and revealing the usefulness of appropriate soil construction methods (Nkrumah et al. 2019d). This process would allow the rehabilitation of rocky or soil-poor areas for a subsequent plant cultivation (*i.e.* mining sites, slopes that need to be flattened).

Agromining Process

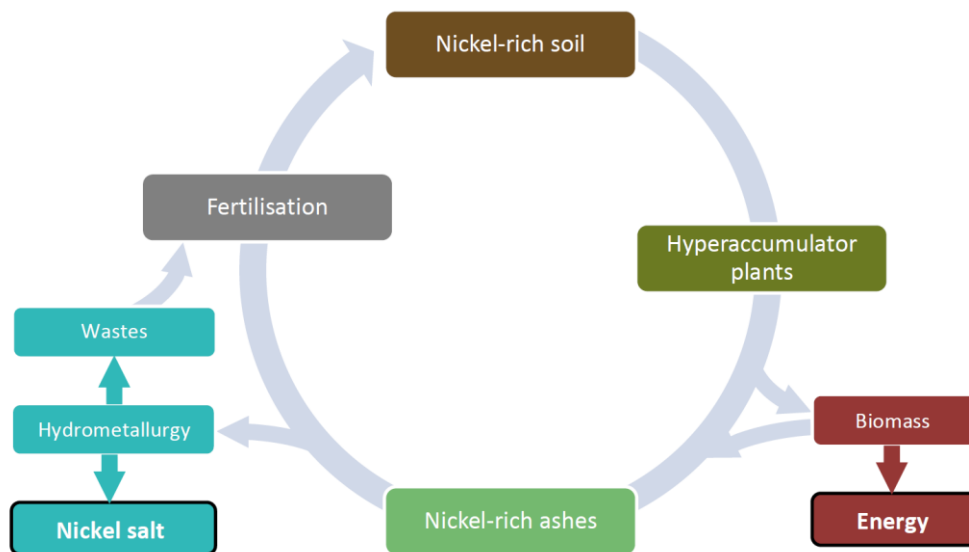


Figure 4 Nickel agromining cycle. Source: <https://life-agromine.com/agromine/>

Process of Ni extraction involves different stages that are continuously optimised. This metal can be recovered from fresh plant biomass or from ashes resulting from plant biomass incineration. Fresh plant extraction is still limited due to the high concentration of organic matter. Reduction to ash is privileged to induce a diminution of transporting and manipulation cost (Zhang et al. 2014, 2016). *Phyllanthus rufuschaneyi* and *Rinorea aff. bengalensis* ashes contains 13% and 5.5% of Ni respectively, (Vaughan et

al. 2016) resulting in much Ni enriched “bio-ores” than those ores from laterite (Simonnot et al. 2018). After the process of washing of ashes, potassium can be recovered efficiency and can be reused (*i.e.* fertiliser) (Barbaroux et al. 2012; Vaughan et al. 2016b; Zhang et al. 2016, Simonnot 2018). Up to 95% of Ni can be recovered from ashes by leaching with sulphuric acid (H_2SO_4) at 60°C. Phosphorus is the unique elemental impurity of nickel hydroxide which can be used in metallurgic processes (Vaughan et al.; 2016). Magnesium, K and P extracted from the previous processes could be recovered and used as a fertiliser for example but the increase of other element concentration such as Ca can rise the recovery cost.

Economics opportunities in Asia-Pacific region

Agromining has remained unimplemented in tropical regions (*i.e.* Southeast Asian region). Economic Nickel agromining could be developed in Sabah (Malaysia), Halmahera (Indonesia) and Palawan (Philippines) (van der Ent et al. 2013; Nkrumah et al. 2016b; 2018b, c). To improve mine rehabilitation operations, Nickel agromining could be implanted in New Caledonia (France), Sulawesi (Indonesia) and Palawan (Philippines). Benefits have been simulated by Barbaroux et al. (2010) with temperate Ni hyperaccumulators. For a surface of 21 600 ha and an extraction of one tonne by ha, total benefit could be upper than 8 millions of dollars Canadian so more than 6 millions of euros.

For *Phyllanthus rufuschaneyi*, Ni yields of 250 kg ha⁻¹ y⁻¹ with a density of five plants per m² are expected, mainly due to its rapid growth. Knowing that the price of Ni has varied from 8 US\$ kg⁻¹ to 22 US\$ kg⁻¹ with an average price of 15 US\$ kg⁻¹ over the last decade, one hectare of tropical agromining could produce up to 3,500 US\$ of Ni per year. The agromining system in Sabah mainly employs manual labour with a relatively low production cost, as it is the case in temperate agromining in Albania. This new type of agriculture could, at the same time, serve as an income source for local communities and, preserve the biodiversity by the cultivation of local endangered species (*i.e.* *P. rufuschaneyi*). However, research on this system is still needed to take into account the principles of agroecology. Ni worldwide demand is increasing due to increasing demand of lithium ion battery. Demand could increase ten-fold by 2030 compared to 2019. Agromining life cycle has a low impact on the environment (Rodrigues et al. 2016). Furthermore, recycling metals through agromining is in adequacy with the principles of circular economy. Predicting tools are still needed to know the longevity of economical agromining on tropics.

F. How to improve agromining ?

Phytoremediation has been studied for more than 20 years. The first field trials aimed to improve Ni yield with Brassicaceae in temperate regions. To improve Ni yields, different methods and strategies can be applied individually or in combination. Organic and inorganic amendments are typically used to improve plant yields. Solutions inspired by agroecology are also conceivable, such as microorganisms inoculation and co-cropping with leguminous to improve N fertility in soils.

a. Amendments

Low fertility of ultramafic soils can limit plant development, which in turn, can reduce metal yields. In temperate regions, numerous studies have been tested the inorganic and organic fertilisation on metal crops. Inorganic fertilisation can enhance the Ni yield in many situations (Alvarez-Lopez et al. 2016; Bani et al. 2007, 2015a). *Alyssum murale* (= *O. chalcidica*) is the model plant studied in temperate regions. In Albania, long-term field trials have been undertaken; biomass production increased from 0.3 to 9.0 t ha⁻¹ and Ni yield increased from 120 kg ha⁻¹ after NPK application (Bani 2009; 2015). In the United States, nutrient addition, irrigation and weed-control, allowed a Ni yield of 400 kg Ni ha⁻¹ using *O. chalcidica* (Chaney et al. 2007). In Spain, *Alyssum pinodasilveae* Ni yield has been improved with NPK fertilisation (Kidd and Monterroso 2005). Test with nitrogen fertiliser induces until a two-fold increase in the biomass produced by *Berkheya coddii* (Robinson et al. 1997). Organic fertilisation also increased *A. serpyllifolium* and *A. bertolonii* Ni yield, but mostly by increasing the biomass yield whereas it reduced Ni concentrations in plant tissues (Álvarez-Lopez et al. 2016a). However, a recent study showed that pig and chicken manure could increase Ni yield in *O. chalcidica* compared to inorganic NPK fertilisation by increasing both plant biomass and Ni concentrations in leaves (Bani and Echevarria 2019). Organic fertilisation could be useful for revegetating soils and could help in recycling metal-rich wastes (Ruttens et al. 2016). Large-scale field trials are essential to validate its usefulness for agromining in temperate and tropical environments (Nkrumah et al. 2016).

The agronomy of the tropical “metal crops” *P. rufuschaneyi* and *R. aff. bengalensis* has been recently studied in a huge pot trial. The first results vary between plant species (Table 1). NPK fertilisation and organic matter amendment had a positive growth effect on *R. aff. bengalensis*. In the case of *P. rufuschaneyi*, only N and P treatments had strong positive growth responses, whereas K additions, and notably organic matter additions, had adverse growth effects with a significant reduction of plant growth.

Calcium and S additions improved Ni uptake of both species without affecting biomass production. The increase in biomass in response to inorganic fertilisation did not significantly reduce Ni concentrations in both species, except for N addition in *P. rufuschaneyi*. On the contrary, organic matter amendments significantly and strongly reduced shoot Ni concentrations of both species and even decreased the biomass of *P. rufuschaneyi*. These results revealed that Ni uptake and growth responses to nutrient input are species-dependent (Nkrumah et al. 2018). In conclusion, Ca, S and P additions seem to be essential in the agronomy of these tropical ‘metal crops’ for economic Ni agromining.

Table 1 Nutrients and organic amendments effects on Ni content, biomass and leaf nickel yield per plant (mg) (Nkrumah et al. 2019a,b,c)

	Biomass		Ni content		Ni yield			
	<i>Rinorea</i>	<i>Phyllanthus</i>	<i>Rinorea</i>	<i>Phyllanthus</i>	<i>Rinorea</i>	<i>Phyllanthus</i>		
	<i>bengalensis</i>	<i>rufuschaneyi</i>	<i>bengalensis</i>	<i>rufuschaneyi</i>	<i>bengalensis</i>	<i>rufuschaneyi</i>		
Control					120		180	
Organic Matter	+	-	-	-	195	+	200	-
Sulphur	+	-	+	+	150	+	300	+
Calcium	+	-	+	+	200	+	325	+
Nitrogen	+	+	/	-	/	+		
Phosphorus	+	+	/	/	/	+		
Potassium	+	-	/	/	/	+		

Harvest method

Many factors influence the propagation management and harvest in temperate and tropical agromining. Planting and harvesting management will be specific to each species, and each location (Nkrumah et al.2019a). In temperate regions, the period of harvesting influences Ni yields in *Alyssum* genera (Bani 2015, Adamidis 2017). In tropical regions, perennial plants are selected in Asian and Pacific regions. Coppicing can be used to make annual harvests, notably for woody species that can accumulate Ni in their trunks. Harvesting could be done at multiple times during one year depending on the plant growth and must be before fruit season. Manual harvesting directly in the secondary forest is also envisaged for non-endangered species, such as *R. cf. bengalensis*.

Soil pH

The uptake of Ni by hyperaccumulator plants is influenced by pH and is optimal within a pH range from 5 to 6.5 in ultramafic soils (Chaney et al. 2007b; Kukier et al. 2004). For Nkrumah et al. (2019d) a reduction in soil pH decreased shoots Ni concentration in *P. rufuschaneyi*, such as is the case for *Alyssum* species (Li et al. 2003; Kukier et al. 2004). Reducing pH would cause a diminution of available elements for plants by changing elements chemical equilibrium in soil system while the plant itself is suited to higher pH. Although shoot biomass decreased with Ni addition, this result shows that a very high available Ni concentrations could have a negative effect on the Ni yields. Conversely, Ni addition ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) in pots increased both the DTPA-extractable soil and the Ni yield for *R. cf. bengalensis*. By now, even for temperate agromining, it is not possible to accurately predict the amount of Ni in shoots from extractable Ni data because of the different soil properties, notably pH. Indeed, more research is required on Ni uptake to develop predictive tools (Nkrumah et al. 2016).

Co-cropping

Co-cropping hyperaccumulator plants with legumes can increase N concentration in soil without inducing potential N leaching in the underground water. Co-cropping with hyperaccumulator and leguminous has been only tested in temperate regions with contrasting results. Chaney (2016) studied co-cropping between *A. murale* and ryegrass (*Lolium perenne*). This grass had a negative effect on Ni yield due to a competition between shoot and roots systems of plants. Jiang et al. (2015) obtained similar results, whereas Saad et al. (2018) had positively effect on Ni yield and plant biomass after testing co-cropping of *A. murale* and a Fabaceae (*Vicia sativa*). Other positive effects were noticed, such as the reduction of soil erosion and weed competition. Further studies are needed even in temperate regions.

Mass propagation

Mass propagation of tropical agromining plants would be different than mass propagation of non-tropical plant, since potential tropical agromining plants are currently perennial plants (notably shrubs or trees). Plants could be propagated by cuttings, directly in field or in pots in a nursery. However, this infrastructure can lead to an extra cost. At the first tropical metal farm, some *P. rufuschaneyi* generate fruits that were collected and germinated in pots in a nursery for three to six months before transplanting. For the pot-test, *Rinorea cf. bengalensis* seedlings were collected directly under “a mother tree”. Use of plants in technology could be a mechanism for conservation of germplasm of rare species.

G. Objectives

Biogeochemical cycles of tropical hyperaccumulator plants in natural forest and under sustained harvesting are still poorly unknown. Litter seems to be able to influx Ni return to the soil, but the study of all the Ni cycles are needed to understand the longevity of economical agromining. Furthermore, the impact of fertiliser on Ni tropical hyperaccumulators are still unknown under field condition, so as the impact of fertiliser on Ni tropical cycles under agromining.

The main objective of this PhD is the characterisation of metal and nutrient cycles of tropical hyperaccumulators plants in both a planted agromining field plot and in a natural stand resulting from forest clearing and regrowth.

To summarise, the objectives that this PhD will address are the following:

- ☼ To study the biogeochemical cycling of a natural forest of *P. rufuschaneyi* in order to assess and evaluate the natural fluxes of Ni in the ecosystem;
- ☼ To manipulate such an ecosystem in order to perform a sensitivity test of the ecosystem for the following flux: litter return to the soil; from no return (export) to a doubling of the return.
- ☼ Based on both the understanding of the role of the litter/Ni biogeochemical cycling in feeding *P. rufuschaneyi* and the plant NPK and Ni requirements, to optimize the cropping system of *P. rufuschaneyi* for Ni agromining.

H. Material and Methods

To have a full control and elemental balance of the systems, different monitoring were done on two plots where stand *Phyllanthus rufuschaneyi*:

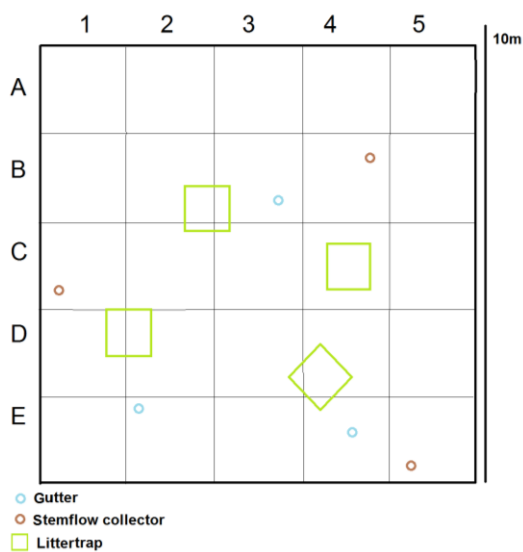


Figure 5 Garras monitored plot

- ☼ Climatic monitoring: t°, humidity, sunlight...
 - ☼ Agronomic monitoring: diameter and growth (every month)
 - ☼ Chemical monitoring (pH, Ni, Co, Ca, K ...): solution and litter
- b. Garras – Natural plot

To study natural cycles of *Phyllanthus rufuschaneyi*, the open forest at Garras, where this hypernickelophore originated, was monitored by a small biogeochemical station. On a plot of 100m² divided in 25 subplots of 4m², litter traps, gutters and stemflow collectors were set up in March 2018. A

destructive plot of 4m² was realized in July 2018 to know the stocks of nutrients and notably of Ni. In November 2019 and in January 2020, the number of *Phyllanthus* by sub-plot was determined and their sizes were measured (height, height of the leaves, height of the first leaves, diameter...). A known volume of soil surfaces were collected at every sub-plots, notably organic horizons. Soil samples were collected at different depths in November too to do chemical and structural analyses.

Pahu – Agromining field

In November 2018, Ten plots of 9m² with four *Phyllanthus* by square meter were established to study the effects of fertilizer on Ni yield. Just after cropping, two different fertilizer treatments were applied. Urea was amended at 120 kg ha⁻¹ on three sub-plots and N+P+K was amended at 37 kg ha⁻¹ on three other sub-plots. The four non-amended sub-plots allowed to know the grow of plants without fertilizer input.

In March 2018, nine sub-plots of 25m² crop with *Phyllanthus* at a density of two plants per square meter (more than 445 plants in total) were instrumented with a geochemical station. In March, the plots were instrumented with a biogeochemical station in which litterfall, stemflow, throughfall, drainage, dry deposits were monitored (Figure 6). The collections of water and litter samples were scheduled every month. Since July 2018, plants from this plot have been harvested every four months and every plant biomass have been collected to know the yield of each plant. A conventional NPK fertilization of 37 kg ha⁻¹ was applied in November 2018, it's the same amount of fertilizer than in plots described in the previous paragraph.



Figure 6 Pahu biogeochemical station instruments

Litter treatments were set up to study Ni biochemical cycle (Figure 7). These treatments were applied in three times, in November 2018, in March 2019 and in June 2019 to study more easily the degradation of the litter with time. On the nine geochemical sub-plots, three received no treatments (A1, A2, B2), three were amended with 1 kg m⁻² of litter only, and three other sub-plots received 2 kg m⁻² of litter so received

twice more litter. At each treatment, litters bag with small mesh were installed ($\pm 0,1\text{cm}$) and were collected at different times (1, 2, 3, 6 months...).



Figure 7 Litter treatment set up

Once collected, pH and conductivity of the water samples were analyzed on site and the elements composition were determined by ICP-OES at LSE. The collection of soil samples (=45) and plants samples (=225 leaves) at time 0 was carried out in March 2018 to characterise the state of the ecosystem before any manipulation treatment takes place. Following soil and plant collections were done every four months. Preparations and analyses of these samples were carried out at LSE in France (Figure 8).

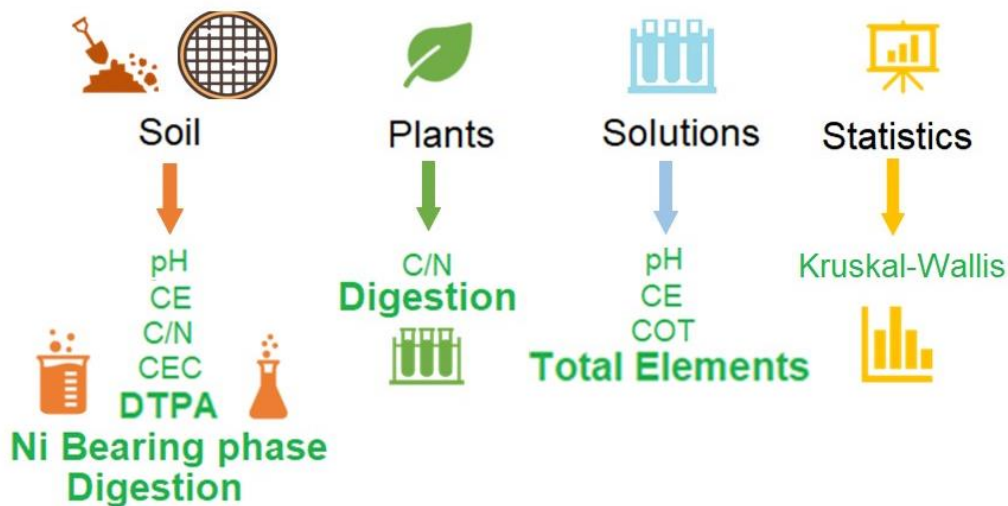


Figure 8 Methods sample analysis

CHAPTER 2: Element budget of a Tropical Ultramafic forest

Biogeochemical cycling of nickel in a high-density stand of the hyperaccumulator *Phyllanthus rufuschaneyi* in Sabah, Malaysia

Romane Tisserand¹, Philip Nti Nkrumah², Antony van der Ent², Sukaibin Sumail³, Bernhard Zeller⁴
Guillaume Echevarria^{1,2}

¹ Université de Lorraine, INRAE, Laboratoire Sols et Environnement, 54000 Nancy, France.

² Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, Queensland, Australia.

³ Sabah Parks, Kota Kinabalu, Sabah, Malaysia.

⁴ INRAE, Centre de Nancy, Ecosystèmes Forestiers, 54280 Champenoux, France

*corresponding author: Guillaume Echevarria (guillaume.echevarria@univ-lorraine.fr)

ABSTRACT

Biogeochemical cycling of nickel (Ni) by tropical hyperaccumulator plants is unknown, although they are suspected to play a major role in recycling of this element in ultramafic ecosystems. In this study we present the biogeochemical cycling of Ni (and other elements) by a tropical Ni hyperaccumulator, *i.e.*, *Phyllanthus rufuschaneyi*, one of the most promising species globally for agromining. The species was studied in a young secondary forest of the state of Sabah (Malaysia) where it occurs as the dominant species on an ultramafic Cambisol. For two years, we monitored a 100-m² plot and collected information on weather, biomass increase, soil fertility, water fluxes to the soil and litter fluxes for elements including Ni. The Ni cycle is mainly driven by internal fluxes, notably the degradation and recycling of the Ni-rich litter. In almost two and a half years, the Ni litter flux corresponded to the total nickel stock of the litter (5.2 g m⁻² year⁻¹). The Ni turnover varies significantly with different accumulation properties of the plant cover, thus pointing out the extreme influence of Ni hyperaccumulators in building up nickel available stocks in topsoils as already suspected in temperate ultramafic systems. Litterfall and throughfall contribute substantially to phosphorus, sulphur and potassium cycling in the ecosystem, throughfall contributing 2-, 220- and 20-fold higher to the respective nutrient fluxes relative to litterfall. The magnesium:calcium ratio far exceeded 1 in the soil, but <1 in the leaves of *P. rufuschaneyi*. These insights should be taken into account when designing tropical agromining as Ni stocks could be more labile than initially thought, and removal of Ni- and nutrients-rich biomass could potentially affect available Ni (and nutrients) for the next cropping season unless soil weathering is a significant contributor to Ni stocks.

Keywords: biogeochemistry; litter; nickel hyperaccumulator plants, nutrient cycling, Southeast Asia; ultramafic soils

A. INTRODUCTION

In tropical rain forest, nutrients are strongly incorporated in plant biomass and the forest floor (Vogt et al. 1986). Nutrient cycles in forest comprise nutrient uptake and storage, litter production and decomposition, nutrient input by atmosphere or weathering, nutrient export by leaching and biological nutrient transformation (Foster and Bhatti 2006). Litterfall represents the main pathway of nutrient transfer from the vegetation to the soil; most nutrients absorbed by plants return to the soil as leaf litter and sometimes as branches and logs (Vitousek 1982, Attiwill and Adams 1993). Litter can maintain the soil fertility by adding organic matter to the soil surface, especially important in humid tropical forests, where the production of litter is globally the highest among all other forest types (Vitousek and Sanford 1986). The dynamics of each nutrient cycle is more or less different depending on the nutrient and the soil under consideration (Vitousek and Sanford 1986).

Ultramafic soils have low prevailing concentrations of essential nutrients, notably phosphorus (P) and potassium (K), but relatively high concentrations of trace elements such as manganese (Mn) and nickel (Ni) (Proctor 2003; Echevarria 2018). In addition, ultramafic soils have imbalanced magnesium (Mg) to calcium (Ca) ratios, which might affect local vegetation (Proctor 2003). Nickel in these soils is largely sequestered in newly formed phases where availability is mainly controlled by Mn-oxides and amorphous or less crystallised iron (Fe)-oxides (Quantin et al. 2008; van der Ent et al. 2018). In tropical ultramafic forest, the vegetation is generally tall forest with relatively low levels of endemism at lower elevations, however, its geoecology remains largely unexplored (Galey et al. 2017). The geochemical peculiarities of ultramafic soils pose an extreme edaphic environment that leads some plants to develop ecophysiological mechanisms to tolerate and accumulate trace elements (Brooks 1987; Proctor 2003). Hyperaccumulator plants accumulate specific trace elements in their shoots far higher than other plants growing on the same soils (Reeves 2003; van der Ent et al. 2013). The threshold for Ni hyperaccumulation is set at 1000 mg kg⁻¹ and to date, Ni hyperaccumulators (> 500 species) form the majority of the known hyperaccumulator plant species (Reeves et al. 2017). Hypernickelophores are Ni hyperaccumulators that can accumulate more than 10 000 mg kg⁻¹ in their leaves (Jaffré and Schmid 1974). These plants occur both in tropical and temperate climates. Some Ni hyperaccumulator species are restricted to ultramafic soils and are termed obligate hyperaccumulators, whereas facultative Ni hyperaccumulator species can occur both in ultramafic and non-ultramafic soils, with only populations on ultramafic soils hyperaccumulating Ni (Pollard et al. 2014). Nickel hyperaccumulation occurs most frequently in the Orders Brassicales and

Malpighiales (Jaffré et al. 2013), notably in Phyllanthaceae family. In Sabah (Malaysia), there are currently 28 known Ni hyperaccumulator species from 10 families and 17 genera (van der Ent et al. 2018).

Tropical Ni hyperaccumulator plants may play significant role in the Ni (and other elements) cycling in the local ultramafic ecosystems, especially in the upper soil horizons, through the uptake of Ni in the roots, litterfall and litter recycling/biodegradation (Echevarria 2018; Ratié et al. 2019). It is suggested that Ni hyperaccumulators may cause an enrichment in Ni concentrations in the surface soils of their local ecosystems (Echevarria 2018), which might provide an ecological advantage for these hyperaccumulating species (Boyd and Martens 1998; Boyd and Jaffré 2001). In the temperate setting, local Ni hyperaccumulator plants directly influence the composition of available and total Ni pools in ultramafic soils (Estrade et al. 2015). The authors suggested that plants, especially Ni hyperaccumulators, may play a major role in maintaining high Ni concentrations in the surface soils during weathering and pedogenesis. However, to-date, the role of plants in Ni (and other elements) in Ni cycling are not yet fully understood (Zelano et al. 2020). Ultramafic soils are widespread in Sabah and Ni hyperaccumulators in the local ultramafic soils are common, both in terms of number of individuals and relative number of species (van der Ent et al. 2015a, 2017). Currently, there is limited information on the dynamics and quantification of the Ni pools in tropical ultramafic soils, especially Sabah.

Insights into Ni (and other elements) cycling in ultramafic systems may enhance our fundamental understanding of (hyperaccumulator) plant-soil interactions in natural ecosystems (Ratié et al. 2019). In addition, these insights may provide useful information required to develop an effective agronomic system for Ni agromining (which involves recovering useful products from harvested biomass of selected Ni hyperaccumulators cultivated on unconventional metal-rich soils) (Chaney et al. 2007; Nkrumah et al. 2016, 2018a, b; van der Ent et al. 2015b). *Phyllanthus rufuschaneyi* is one of the tropical hyperaccumulator plants with desirable traits for economic Ni agromining ('metal crop') in the tropics (Nkrumah et al. 2019a, b, c, d). This species accumulates exceptional concentrations of Ni in its shoots, with up to 2.5 wt% in old leaves and 1.2 wt% in twigs (Bouman et al. 2018; van der Ent et al. 2015a; van der Ent and Mulligan 2015). In addition, *P. rufuschaneyi* has rapid re-growth after coppicing, pest resistance and well adapted to open areas (Nkrumah et al. 2019a). The biogeochemical cycling in the native habitats of this recently discovered 'metal crop' could broaden our understanding on the agronomic systems required to develop sustainable Ni agromining, and the long-term effect of agromining on the local ecosystem. A large pot

trial has been recently undertaken to test the effects of soil organic and inorganic amendments on selected tropical ‘metal crops’, *P. rufuschaneyi* and *Rinorea cf. bengalensis* (Nkrumah et al. 2019b, c, d). In addition, the first tropical ‘metal farm’ has been set up in Sabah, employing *P. rufuschaneyi*, with the aim to evaluate the viability of tropical Ni agromining at field-scale (Nkrumah et al. 2019a). The authors supposed a potential Ni yield of 250 kg Ni ha⁻¹ y⁻¹, but this yield needs to be confirmed under real field conditions. In addition to exceptionally high potential Ni uptake, *P. rufuschaneyi* accumulates remarkable concentrations of K and Ca in the shoots, despite the extremely low concentrations of these elements in the local ultramafic soils (van der Ent and Mulligan 2015). The high concentrations of K and Ca in the shoots of *P. rufuschaneyi* may be eventual contaminants during the Ni recovery process of the harvested biomass (Vaughan et al. 2017). Therefore, understanding the soil-plant interactions could help develop agronomic systems that limit the uptake of Ca and K while enhancing Ni yield and recovery efficiency of *P. rufuschaneyi*.

The aim of this study was to investigate the dynamics of the soil-plant elemental cycling, focussing on Ni, in the native habitats of the tropical ‘metal crop’ *P. rufuschaneyi*. The primary questions this study sought to address are two-fold: i) how do Ni hyperaccumulator plants modify Ni (and other elements) cycling in a tropical lowland forest dominated by Ni hyperaccumulators, and ii) what are the main mechanisms that dominate the Ni (and other elements) fluxes at the ecosystem level.

B. MATERIALS AND METHODS

a. Study site and sampling

The study site is located in North Borneo, in the Malaysian state of Sabah, at Garras Hill, in a young secondary forest regrown after a fire that took place 7–8 years before the study started (latitude 6.088°N, longitude 116.798°E) (Figure 9). The site is established on an ultramafic Hypereutric Cambisol where *P. rufuschaneyi* grows spontaneously. This shrub, restricted to only two nearby localities (*i.e.*, Bukit Hampuan and Lompoyou Hill), may accumulate up to 2.5 %Wt Ni in leaves (Bouman et al. 2018). A plot of 100 m², divided into 25 subplots of 4 m², was set up. From March 2018, all the *P. rufuschaneyi* species growing in the experimental site were monitored using a small biogeochemical station including gutters, stemflow collectors and litter traps randomly positioned in the plot (Figure 9). A 1-m² litterfall collector of and three smaller 0.25-m² litter collectors of were installed 50 cm above ground level. Litterfall and

water samples were collected every month from March 2018 to January 2020. In July 2018, a destructive plot of 4 m² was setup at the north-east of the main plot, to determine the stock of Ni and other elements in the biomass and the different soil compartments (Litter and Soil horizons). Three soil cores were extracted (Figure 9), one soil core was extracted from the destructive plot and two others were collected uphill and downhill outside the geochemical plot. Soil samples were divided according to the number of visible horizons (organic horizons: O, top-soil: A1₁ and A1₂, weathering horizon: B and transitional horizon: BC) (Figure S1 and S2). Known volumes of forest floor (O) and surface soils (A1₁) were collected from each sub-plot in November 2018 and from the sub-plot with relatively high Ni in January 2020 (O). The organic horizon is a thin fragmented horizon (1 to 3cm) that can be classified as a Moder; its boundary with mineral soil was consistently distinct and its components were classified according to plant species composition and their decomposition (O = OL+ OF; OL= litter and OF= fragmented). In November 2018 and 2019, the number of *P. rufuschaneyi* in each plot was counted and growth parameters were measured in each of the sub-plots (plant height, the number of leaves, the height of the first leaves, plant diameter, etc). Furthermore, in January 2020, nine plants from the sub-plot containing more Ni were collected randomly along with their rhizosphere soils.



Figure 9 Overview of the biogeochemical plot and the monitoring instruments (gutter, litter trap and stemflow collector) in an ultramafic ecosystem dominated by the Ni hyperaccumulator *Phyllanthus rufuschaneyi* in Sabah, Malaysia

Soils analyses

Soil samples were analysed at the Lse lab at the University of Lorraine in France. Soil densities were determined by using a metal cylinder of a known volume and then by heating subsamples to 105 °C until a constant weight was reached. Physico-chemical analyses were performed on air-dried and sieved soil (< 2 mm) samples (according to NF ISO 11464). Soil pH was obtained in a 1:5 soil:water mixture after 1-hr equilibrium time on an end-over-end shaker (according to NF ISO 10390). Total and organic C and N were quantified using a CHNS analyser (according to NF ISO 10694 and ISO 13878). The cation exchange capacity (CEC) was determined spectrophotometrically (according to ISO/DIS 23470) by extraction with a hexaamminecobalt(III) trichloride solution. Exchangeable trace elements were extracted with DTPA (diethylene triamine pentaacetic acid), 1:2 ratio of soil/DTPA solution. To determine the pseudo-total elements content, 0.5 g of each soil sample was digested using Aqua Regia (2 mL 70% nitric acid and 6 mL 37% hydrochloric acid per sample). After 16 hrs, samples were heated at 105 °C for 120 min and the resulting solutions were diluted to 50 mL with ultrapure water. The digests/extracts were filtered (< 0.45 µm), and then analysed with ICP-AES (Thermo Fisher iCAP 6300 Duo). Selective oxide dissolutions were performed according to the method described in Alves *et al.* (2011). The amorphous metal oxides were extracted by the ammonium oxalate–oxalic acid method, amorphous and crystallised oxides were extracted using the citrate–dithionite method and hydrous manganese oxides were extracted with hydroxylamine hydrochloride in nitric acid.

b. Plants and litter analyses

Roots were washed with ultrapure water and tetrasodium diphosphate (shaken for 24 h). Roots and shoots were dried at 40 °C for 24 h, and separately powdered using a ball mill. Leaves and litterfall were separated into two groups, *Phyllanthus* and *non-Phyllanthus*. Total and organic C and N were quantified with a CHNS combustion analyser (Vario Micro Cube, Elementar, Germany). To determine the concentration of the pseudo-total elements, ~0.050 g of each sample was digested with 2 mL HNO₃ and 1 mL H₂O₂. After 16 hrs, samples were placed in a DigiPREP® system for 120 min at 95 °C and the resulting solutions were diluted to 10 mL with ultrapure water and then filtered at 0.45 µm before analysis with ICP-AES (Thermo Fisher iCAP 6300 Duo). Elemental concentrations in litter are measured in the same way.

c. Water analyses

After collection, every month, throughfall and stemflow samples were filtered at 0.45 µm and were then stored refrigerated at 4°C in Sabah until analysis. Samples for total element analyses were acidified with ultrapure HNO₃ before analysis with ICP-AES (Thermo Fisher iCAP 6300 Duo).

Calculation of the elements flux, litter decomposition and residence times

Water fluxes (throughfall and stemflow) and litterfall were calculated from March 2018 to January 2020. Total rainfall (TR) and atmospheric deposition (deposit of airborne materials = DD) were calculated by using the results of a weather station installed in a nearby large ultramafic field in Pahu, Sabah, Malaysia (6°7'49"N; 116°46'22"E). During this period, total rainfall was 3720 mm, which is ~2180 mm on average per year, and a mean temperature of 24.9 ± 0.6 °C. Stemflow flux was calculated considering the average density of *P. rufuschaneyi* to be two plants m⁻² within the 100-m² monitored plot. Throughfall and stemflow comprise 92.5% and 7.5%, respectively, of the local gross rainfall. By using rainfall data of the nearby Pahu site, a potential interception by canopy of 8.5% was calculated. Total nutrient input (It in g m⁻² y⁻¹) is calculated by adding the quantity of nutrients in litterfall (Lf), stemflow (Sf) and throughfall (Tf):

$$It = Sf + Lf + Tf - DD \text{ [g m}^{-2} \text{ y}^{-1}] \quad (1)$$

In order to determine the leaf mass, organic horizon weight of November 2018 (A₀M_{t0}) was compared to organic horizon weight of January 2020 (A₀M_{t1}). Leaf litter disappearance (LLD in g m⁻²) was calculated by an equation adapted from Golley et al. (1978), which is the difference between the sum of leaf litter mass (LLM) at the beginning of the period and leaf litterfall (LLF) during the period, and LLM at the end of the period:

$$LLD = LLM(t_0) + LLF - LLM(t_1) \text{ [g m}^{-2}] \quad (2)$$

Only a litter layer mass loss at a precise time could be calculated because we did not know the stability of this layer over time:

$$K_L: \text{ annual LLF / average annual LLM} \quad (3)$$

Table 2 Maximum, minimum and average elemental concentrations (mg kg⁻¹) of the soil profile of a *Phyllanthus rufuschaneyi* dominated ultramafic ecosystem in Sabah, including the forest floor and organo-mineral horizons (OL= organic horizon; OF= fragmented horizon, A11= surface horizon), as well as subsoil (B and C) horizons. Total organic carbon (Corg) and total nitrogen (N) are given in g kg⁻¹. Results correspond to sub-plot data collection in November 2018.

		METALS								NUTRIENTS								
Horizons		mg kg ⁻¹								mg kg ⁻¹						g kg ⁻¹		
		Al	Co	Cr	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	Na	P	S	Corg	N	
Monitored plot – Topsoil	OL (Phyllanthus)	Max	--	--	--	--	1800	53 000	390	18 000	8500	4700	--	960	1800			
		Min	--	--	--	--	1000	20 000	170	6200	5500	1200	--	200	1000			
		Average	--	--	--	--	1300	32 000	250	9500	2900	3000	--	360	1300			
	OF	Max	1500	70	580	18	13 600	430	11 200	120	17 600	1400	15 000	60	600	1450	490	16.2
		Min	200	10	20	9	500	70	1700	40	7100	700	4100	10	240	770	290	8.4
		Average	900	30	260	12	5900	210	5700	80	10 300	900	9100	30	440	1070	400	12.7
	A1 ₁	TOTAL	11 300	120	2600	15	66 500	1160	2500	40	3000	400	53 900	30	310	740	186	8.2
		DTPA	0.1	1.4	--	1.9	100	15	100	0.56	--	20	140	2.3	3	--		
	Destructive plot - Soil cores	A1 ₁	TOT	13 300	220	3300	27	89 000	2400	1700	70	4800	390	54 700	40	270	400	
DTPA			--	1.3	--	0.8	30	30	90	1.4	--	13	200	--	--	7.0		
CEC			--	--	--	--	--	18	24	--	2100	180	2600	--	--	--		
A1 ₂		TOT	17 600	240	4100	22	113 000	3000	1900	80	1100	140	72 700	23	140	270		
		DTPA	--	2	--	1.8	80	40	90	1.0	--	14	600	3.6	--	6.0		
		CEC	--	--	--	--	--	8.0	12	--	550	60	2600	--	--	--		
B		TOT	19 800	420	4900	27	141 000	4300	2400	90	900	90	79 500	30	100	160		
		DTPA	--	2.1	0.1	1.9	90	60	80	0.7	--	10	1000	5.9	--	6.0		
		CEC	--	--	--	--	--	9.0	11	--	180	20	2100	--	--	--		
C		TOT	18 300	200	2900	38	121 000	1800	2300	90	240	40	85 400	--	30	70		
		DTPA	--	0.7	--	1.9	60	16	50	0.5	--	1.8	1500	2.9	--	2.1		

For an element, its stock was calculated by multiplying the mass of the compartment W_{stock} (kg m⁻²) by its concentration in this element C (g kg⁻¹):

$$Stock = W_{stock} * C_{tot} [g m^{-2}] \quad (4)$$

For an element, the mean residence time (RT) during litter decomposition was calculated using the equation described by Vogt et al. (1986):

$$RT = stock / It [y^1] \quad (5)$$

This result needs to be used carefully because this calculation does not take into account root input, however, it can already give an idea of nutrients turnover inside a hypernickelophore forest.

Statistical treatment

Statistical analyses were conducted with R software version 3.6.2 (2019-12-22) and XLstat (version 2019.3.2). QGIS version 3.14.15 was used to plot the soil and plant parameters. Kruskal-Wallis analyses were performed to compare the stocks of elements in the different compartment and to know the impact of the time on the Ni flux. The level was set at 0.05 for each statistical evaluation.

c. RESULTS

a. Composition and stocks of Ni in the plot

The soil had relatively low concentrations of nutrients, P (30–270 mg kg⁻¹), S (65–400 mg kg⁻¹) and K (35–390 mg kg⁻¹), compared to elevated concentrations of Cr (2910–3290 mg kg⁻¹), Mn (1800–2400 mg kg⁻¹), Fe (8.9–12.1 g kg⁻¹) and Ni (1710–2990 mg kg⁻¹) (Table 2), which is typical of ultramafic soils. Moreover, the Mg concentrations were extremely high (5.4–8.5 g kg⁻¹), compared to relatively low Ca concentrations (240–4880 mg kg⁻¹). Along the soil profile, the total concentrations of Mg and Ni increased with soil depth, whereas P, S, K and Ca had an opposite trend (Table 2). The total Ni stock in the soil was extremely high (12 000 kg ha⁻¹), ~60-fold higher than that of the forest floor and the plants (Table 3). The Mg stock in the soil was also relatively high (38 000 kg ha⁻¹) compared to Ca (1000 kg ha⁻¹) (Table 3). Manganese oxides were not the principal carrier phase of Ni in the soil profile, but Ni was associated with the amorphous oxides in the topsoil, and crystallised oxides in the B and C horizons (Figure 10). The DTPA-extractable Ni concentrations decreased with soil depth; ~90 mg kg⁻¹ in the topsoil but two-fold

lower in the C horizon (Figure 10). The decrease in the DTPA-extractable Ni concentrations along the soil profile corresponded with an increase in the crystallised oxides-associated Ni concentrations (Figure 10). In the topsoil, the A₁₁ and A₁₂ horizons had similar DTPA-extractable Ni concentrations, whereas Ni associated with amorphous oxides was higher in the A₁₁ horizon, compared to the A₁₂ horizon (Figure

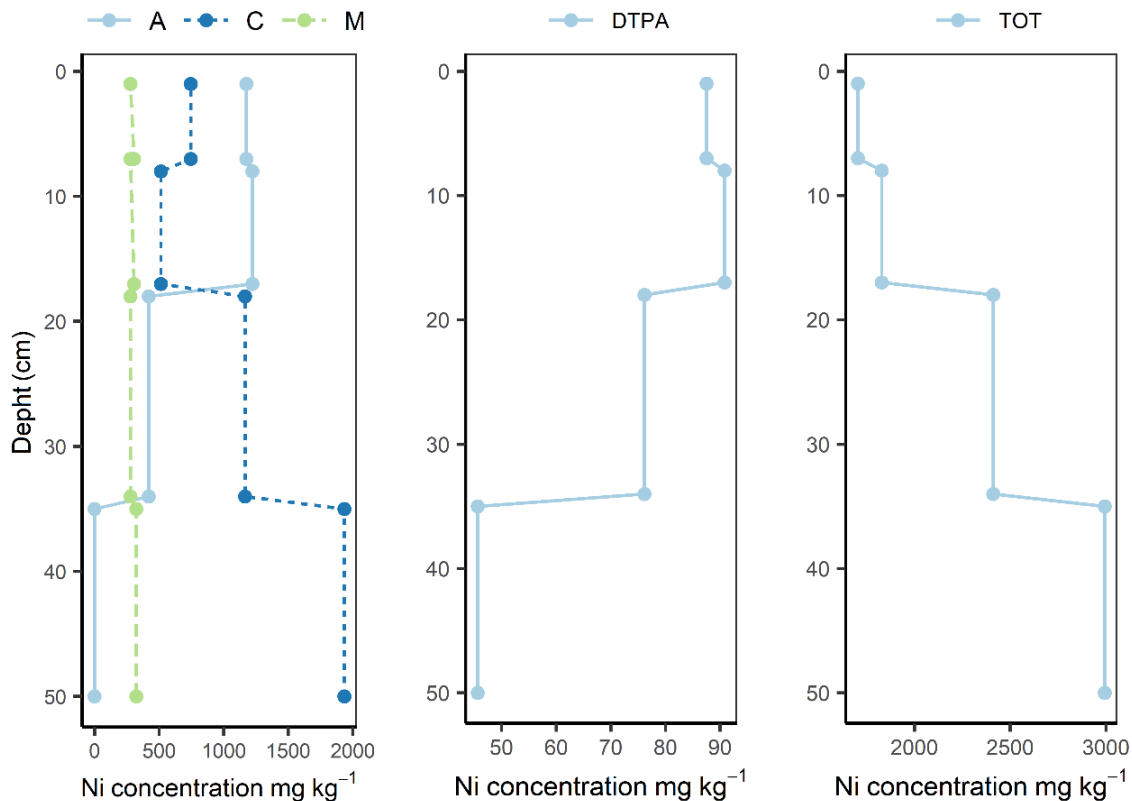


Figure 10 Exchangeable, DTPA-extractable and total Ni concentrations (mg kg^{-1}) as of July 2018 in the soil profile of the studied ecosystem (A = amorphous oxides, C = crystalline oxides, M = manganese oxides, TOT= pseudo-total).

10).

The organic horizon had a leaf litter disappearance (LLD) of $0.45 \text{ kg m}^{-2} \text{ y}^{-1}$ and a litter decomposition rate (k_L) of 1.1; it is indicative of a rapid turnover. For Ca, no impact of litter degradation on its concentration was notable for the organic horizon (Table 2). A loss of Ni, Mn and K was visible between the non-fragmented litter (OL) and the fragmented litter (OF). However, no Ni enrichment due to organic horizon degradation was visible on A₁₁ horizon. The stock of Ni in the forest floor corresponded to 160 kg ha^{-1} (Table 3). The other significant stocks were Ca (290 kg ha^{-1}) and Mg (260 kg ha^{-1}).

The Ni concentrations in the various plant fractions of *P. rufuschaneyi* are shown in Figure 11. The leaves had the highest Ni concentrations, (1.8 wt.% Ni on average, with a maximum of 3.5 wt.% Ni), whereas the trunk had the lowest Ni concentrations (0.4 wt.% Ni on average, with a maximum of 1.0 wt.%). The root was relatively enriched in Ni compared to the twigs and trunk (Figure 11). The trunk

constitutes 71% of the aboveground tree biomass, whereas twigs and leaves make up 22% and 7%, respectively. The Ni stock in the *Phyllanthus* stand was 33 g m⁻² in November 2018 (Table 3), with 19 g m⁻² in aerial biomass out of which 6.5 g m⁻² was in the leaves. The standing tree biomass of the plot had 1.9 kg of Ni (*i.e.* 190 kg ha⁻¹), which was relatively higher than K (1.4 kg) but lower than Ca (2.4 kg).

Table 3 Mean elemental stocks (g m⁻²) in the native habitat of *Phyllanthus rufuschaneyi* occurring in Garras, Sabah, in July 2018.

		Al	Co	Cr	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	Na	P	S
Stock (g m ⁻²)	Soil	910	150	940	20	6200	150	1200	40	100	10	3800	20	80	120
	Forest floor	2.6	0.08	0.7	0.03	17	0.6	16	0.2	29	2.7	26	0.1	1.3	30
	Aerial biomass	--	0.48	0.01		0.4	0.07	19	0.22	24	14	1.9	0.04	1.6	0.9

Plant and forest floor stocks were calculated with data obtained from the destructive sampling subplot and soil stocks were calculated with soil cores results.

Interactions between nutrients, metal and plant growth

The most abundant species in the experimental plot was *P. rufuschaneyi*, with more than 200 individuals in November 2018; the largest individual had a diameter of 8.2 cm (Figure 12). For the destructive plot, the largest tree had a diameter of 10 cm and a height of ~6.0 m. The vegetation of this plot was heterogeneous, with *P. rufuschaneyi* being the dominant species in some sub-plots, whereas it was the minority species in other sub-plots where competition with other species appeared. There was significant spatial variation in the soil Ni concentrations in the plot (Figure 12). The region with the relatively higher total and DTPA-extractable Ni concentrations correlated strongly with the occurrence of matured *P.*

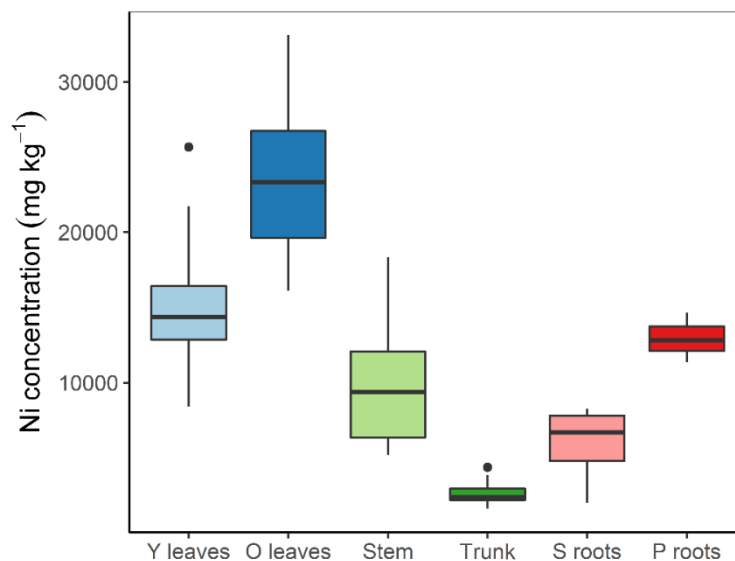


Figure 11 Nickel concentrations in the different parts of *Phyllanthus rufuschaneyi* in November 2018 in mg kg⁻¹.

rufuschaneyi stand. Moreover, the Ni concentrations in the forest floor litter were relatively higher in the subplots within this

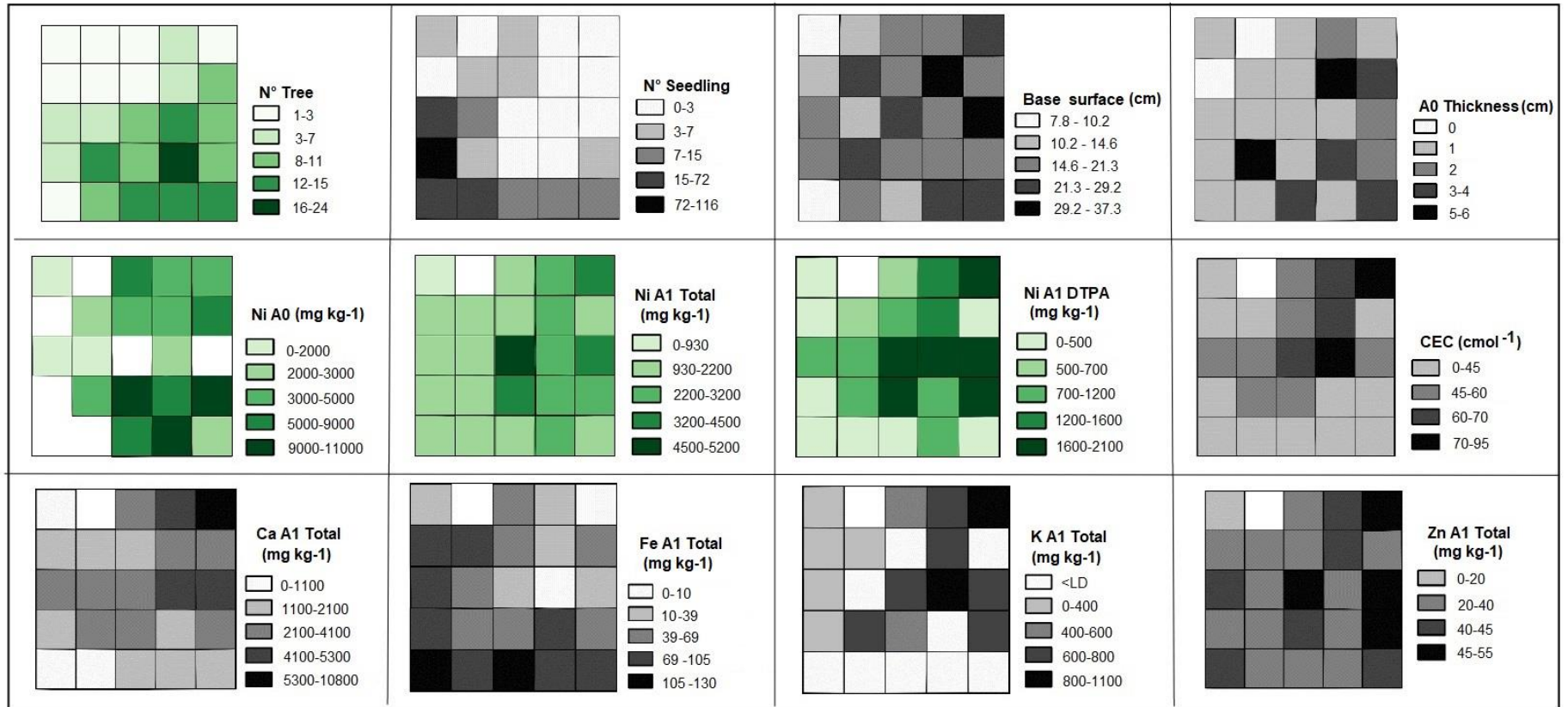


Figure 12 Spatial variations of tree parameters and elemental concentrations in sub-plot in November 2018. Base diameter is given in cm, thickness of the organic horizon is given in cm, CEC of A1 is given in cmol^{-1} , and total and DTPA-extractable elemental concentrations are given in mg kg^{-1} .

region. However, this matured *P. rufuschaneyi* stand dominated and Ni-rich region had relatively low occurrence of *P. rufuschaneyi* saplings (Figure 12, S3), with negative correlation between the number of *P. rufuschaneyi* saplings and the DTPA-extractable Ni concentrations (R^2 -0.42, $p < 0.05$). The total amount of organic matter was not correlated with the DTPA-extractable Ni and was positively correlated with the total Ni concentrations (R^2 0.5., $p < 0.05$). There were no correlations between the Ni concentrations of the litter and its thickness. However, the total and the DTPA-extractable Ni concentrations in the A1₁ horizon negatively correlated (R^2 -0.50, p 0.017) with the weight of organic horizon.

Table 4 Average elements flux and residence time of the hyperaccumulator forest in Garras Hill, Sabah from March 2018 to January 2020 in $g\ m^{-2}\ y^{-1}$ (* results from the biogeochemical station in Pahu).

	Al	Co	Cr	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	Na	P	S
Atmospheric Deposition*	0.02	--	--	--	0.02	0.01	0.02	0.09	0.7	0.25	0.3	0.4	0.01	17
Throughfall	--	--	--	--	--	0.02	1.7	--	2.7	7.8	2.6	1.3	0.13	33
Stemflow	--	--	--	--	--	--	0.024	0.01	0.07	0.2	0.03	0.05	0.01	0.96
Litterfall	--	0.01	--	--	--	0.01	5.8	0.05	1.8	0.36	0.5	--	0.06	0.15
Total nutrient return/ Flux	--	0.01	--	--	--	0.02	7.5	-0.03	3.8	8.1	2.8	0.9	0.2	17
Contribution by litterfall (%)	--	100	--	--	--	50	82	--	47	4.4	19	0	30	<1
Residence time (year)	--	--	--	--	--	3.5	2.6	--	6.4	1.8	0.7	0.04	8	0.05

Element fluxes

Plant stand had significant effect on the Ni concentrations in the throughfall and stemflow (Figure 5, Table 4). There were no statistical differences in the elemental concentrations between throughfall and stemflow, except Mg, which was relatively higher in the throughfall ($0.8\ mg\ L^{-1}$) (Table S1). The monthly variation of Ni concentrations in the throughfall and stemflow was significant ($p < 0.05$) (Figure 5). Return by throughfall represented more than 22% of Ni fluxes back to the soil ($1.7\ g\ m^{-2}\ y^{-1}$) and almost all of S flux ($33\ g\ m^{-2}\ y^{-1}$). The return of elements by stemflow was relatively low compared to throughfall and litterfall, but represented a localized proportion of element recycling, notably for P.

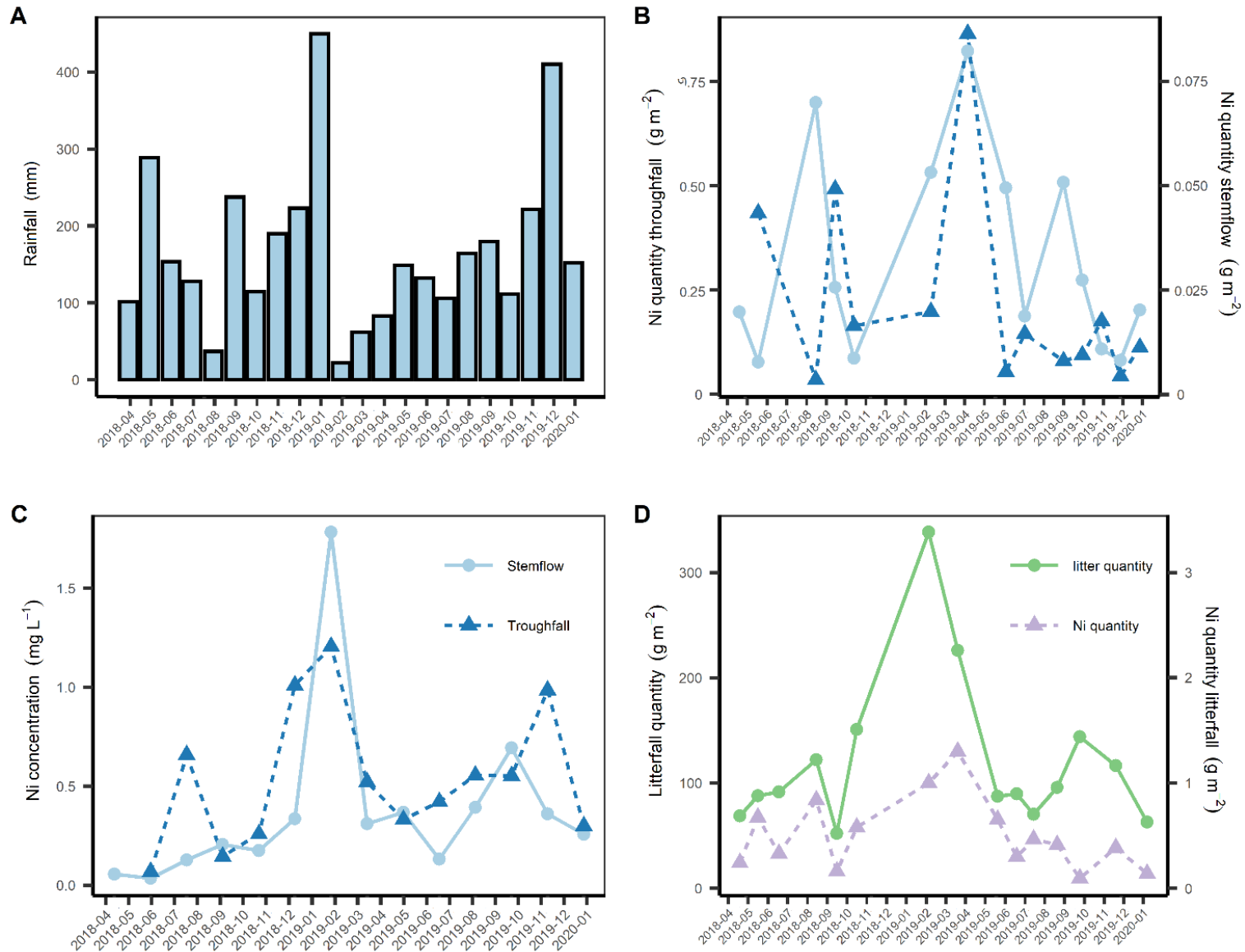


Figure 13 Rainfall and nickel fluxes in throughfall, stemflow and litterfall from April 2018 to January 2020. A: Rainfall (mm); B: Ni quantity in stemflow and throughfall (g m⁻²); C: Ni concentration in stemflow and throughfall (g L⁻¹); D: Litter quantity and Ni quantity in litterfall (g m⁻²).

Litterfall from April 2018 to January 2020 is shown in Figure 14. No significant difference was found between traps. However, the monthly variation in the amount of litter collected was significant ($p < 0.05$). The amount of litter in September 2018 was three times higher than in October. The period from middle February to end of March 2019 was the period with the highest amount of litterfall per day ($130 \text{ g m}^{-2} \text{ period}^{-1} = 5 \text{ g m}^{-2} \text{ day}^{-1}$), which was also the driest period. This period also had the highest amount of Ni litter flux (1.6 g m^{-2}) and the period with the highest Ni concentrations, 1.2 mg L^{-1} and 1.7 mg L^{-1} , respectively, in the stemflow and throughfall. There was no correlation between rainfall and litterfall. A litterfall of 1.5 kg m^{-2} (i.e. 15 t ha^{-1}) can be expected in only one year, which would correspond to an input of more than $5.8 \text{ g Ni m}^{-2} \text{ yr}^{-1}$ (i.e. $58 \text{ kg Ni ha}^{-1} \text{ yr}^{-1}$) and $1.8 \text{ kg m}^{-2} \text{ yr}^{-1} \text{ Ca}$.

During leaf senescence, *P. rufuschaneyi* does not relocate Ni from old leaves to other shoot organs, a non-statistical enrichment of 25% of Ni was noted (Table 5; Figure S4). However, relocation was visible for some nutrients, notably the concentrations of K and P decrease by 80% and 65%, respectively, in the litterfall compared to the old leaves.

Table 5 Differences between average elemental concentrations (mg kg^{-1}) in the leaves and litterfall of *P. rufuschaneyi* from March 2018 to January 2020.

mg kg^{-1}	Mn	Ni	Zn	Ca	K	Mg	P	S
Leaves	110	18 000	190	11 000	13 000	3600	940	990
Litterfall	70	24 000	190	9800	2600	2900	320	860
Difference	-35%	+25%	0%	-10%	-80%	-20%	-65%	-15%

b. Nickel (and other elements) biogeochemical cycling

Nickel inputs through atmospheric deposition had minor contributions to the Ni budget in the tropical hyperaccumulator forest ecosystem (Figure 14). The Ni cycle was mainly driven by internal fluxes, notably by the degradation and recycling of the litter. In almost two and a half years, the Ni litter flux corresponded to the total Ni stock of the litter (Figure 14). The Ni stock of the litter was rapidly “renewed” and appeared to degrade quickly. In addition, plant litter also drove the nutrients cycling in the studied ecosystem, contributing to more than 80% of the total nutrients in the topsoil. Sodium and S cycles appeared to be particularly fast with a residence time of approximately two and three weeks, respectively. In contrast, Cr, Cu and Zn were below limit of ICP-AES detection and so are retained in the canopy.

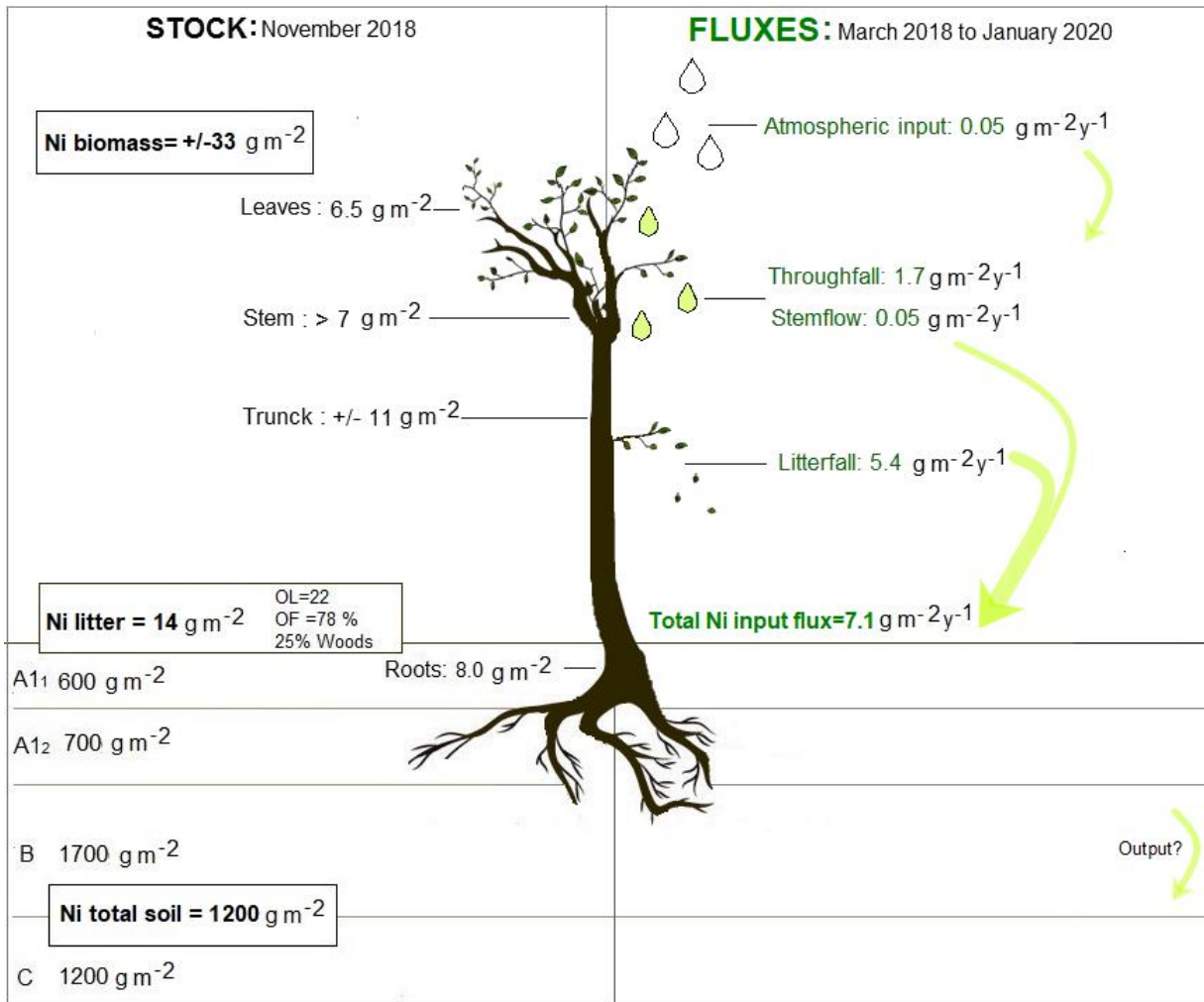


Figure 14 Nickel biogeochemical cycle of a *Phyllanthus rufuschaneyi* dominated forest in Sabah from March 2018 to January 2020. Stocks in g m⁻² and fluxes in g m⁻² y⁻¹.

Agronomic parameters and stock evolution

The Ni concentration and stock evolution over one year, between November 2018 and November 2019 were compared (Figure 15). Nickel concentrations in leaves and surface soils did not vary in that period. However, DTPA-Ni changed considerably, from 1560 mg kg⁻¹ in November 2018 to 600 mg kg⁻¹ in January 2020. The DTPA-extractable Ni of the rhizosphere soil was significantly lower than the topsoil (80 mg kg⁻¹) ($p < 0.01$). Furthermore, total Ni concentrations in the organic horizon increased in one year from 5000 mg kg⁻¹ to ~7000 mg kg⁻¹. The weight of this horizon also increased from 6.9 kg m⁻² to 14.3 kg m⁻², leading to a three-fold increase in Ni stock from 37 g m⁻² to 100 g m⁻² in the forest floor.

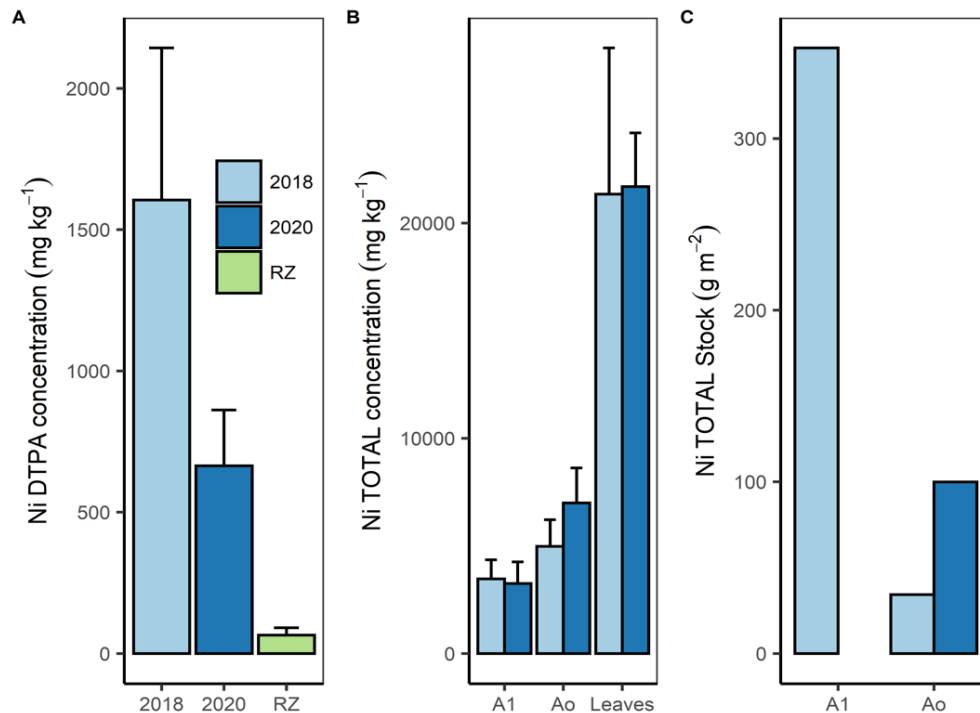


Figure 15 Variations of Ni concentrations and stocks between November 2019 and January 2020. A: Ni DTPA concentrations of surface and rhizosphere soils in mg kg⁻¹ (Rz: rhizosphere soil); B: Ni concentrations in leaves and surface horizons in mg kg⁻¹; C: Ni stock in organic horizon and surface soil in g m⁻².

D. DISCUSSION

The remarkable foliar elemental concentrations of Ni hyperaccumulators coupled with the peculiar geochemical properties of ultramafic soils suggest that these systems are attractive models for biogeochemical cycling investigations (Echevarria 2018; van der Ent et al. 2017; Ratié et al. 2019). The strong Ni uptake and hyperaccumulation by *P. rufuschaneyi* is indicative of the strong role this species may play in the Ni cycling in the local ultramafic ecosystem. The roots are relatively enriched in Ni compared to the DTPA-extractable Ni concentrations in the soil (Figure 10Figure 11), which suggest a highly efficient Ni uptake mechanism. Hyperaccumulators take up Ni from the same labile pool as non-hyperaccumulators but at a faster rate which may lead to depletion of the phytoavailable fraction (Echevarria et al. 2006; Centofanti et al. 2012; Deng et al. 2014). It is possible that Ni from the non-labile pools replenishes the labile pool over time (Centofanti et al. 2012), but this is a rather slow process which depends on the local buffering system (Massoura et al. 2004). Recently, the role of Ni-rich litter in replenishing the labile Ni pools or even the total Ni concentrations in the topsoils have been reported (Bani et al. 2014; Estrade et al. 2015; Echevarria 2018; Ratié et al. 2019). This study shows enrichment in DTPA-extractable Ni concentrations in the topsoil relative to subsoil horizons despite an opposite trend in total Ni concentrations (Table 3). This enrichment

in the Ni phytoavailable fraction in the topsoil horizon suggests an efficient Ni recycling from the decomposition of Ni-rich litter (Estrade et al. 2015; Ratié et al. 2019). The leaves of *P. rufuschaneyi* accumulates exceptional concentrations of Ni (mean: 2.0 wt%) (Figure 11). The Ni concentrations in the litterfall were relatively higher than the leaves, suggesting limited Ni transfer from senescent leaves to developing tissues. Notably, the highly enriched litterfall of *P. rufuschaneyi*, as high as ~3.0 wt% Ni, contributes significantly (77%) to the observed local total Ni input flux (Figure 14). Moreover, the litter in the local ecosystem hosts a significant Ni stock relative to the Ni stock biomass (Figure 14). Therefore, the large amount of litter generated ($1.1 \text{ kg m}^{-2} \text{ y}^{-1}$) and the rapid decomposition rates, coupled with the inherently high Ni concentrations ensure efficient incorporation of readily available Ni in the topsoil. This implies Ni recycling from the Ni-rich litter may replenish the labile pool faster than non-labile pool supply through weathering. Unfortunately, this study could not evaluate the amount of Ni released by mineral weathering and how much is annually leached by draining waters.

Soil Ni extractability is mainly influenced by the nature of Ni bearing mineral phases and mineralogy (Chardot et al. 2007). In this study, the Ni in the topsoil is associated with amorphous Fe-oxides, whereas that in the B and C horizons are associated with crystallised Fe-oxides thus less available (Figure 10). Whereas Ni extractability is high in soils with amorphous Fe-oxides bearing phases, that in highly crystallised bearing phases is low (Raous et al. 2010, 2013; Echevarria 2018; van der Ent et al. 2018). In fact, the profiles of Ni associated to amorphous Fe oxides and DTPA-extractable Ni are parallel curves. This implies that the Ni release from the Ni-rich litter in the topsoil is relatively available for plants because of the prevailing local Ni bearing phase which is able to capture the released Ni. The total Fe concentrations in the B and C horizons are relatively higher than the topsoil, which might further explain the relatively low Ni extractability in these horizons despite relatively higher Ni stocks compared to the topsoil horizons (Table 2Table 3; Figure 14).

The total P, S and K concentrations in the ultramafic soil are depleted compared to non-ultramafic soils in Sabah (van der Ent et al. 2016). However, *P. rufuschaneyi* accumulates exceptional concentrations of K in the leaves, $> 1 \text{ wt\%}$ (Table 5). The physiological role of K in *P. rufuschaneyi* is yet to be investigated, but K is known for its turnover effect. The litterfall is strongly depleted in K relative to the leaves, suggesting strong phloem redistribution to developing tissues before leaf abscission and/or leaching of K during leaf senescence. The litter is, however, K enriched compared to the soil, and litter decomposition may result in K

enrichment in topsoil relative to subsoil (Table 3). Phosphorus and S exhibit similar cycling pattern as K. Phosphorus, K and S may affect the primary productivity of the local ecosystem but the limiting element is yet to be investigated. However, P availability constrains the productivity of most lowland tropical forests (Vitousek 1984). Taken together, P, S and K recycling through leaf litter decomposition may play a significant role in maintaining the primary productivity on the nutrient impoverished ultramafic soils. The Mg:Ca ratio in the soil is extremely high, which is typical of ultramafic soils and may be an indication of the so-called 'serpentine syndrome' (Proctor 1970, 2003). Whereas Ca concentrations are higher in the topsoil relative to the C horizon, Mg has an opposite trend. The Mg:Ca ratio increases from 545:50 (~10 mg kg⁻¹: mg kg⁻¹) in the topsoil to 855:2.5 (~355 mg kg⁻¹: mg kg⁻¹) in the C horizon (Table 3). The leaves of *P. rufuschaneyi* have a Mg:Ca ratio of 18:55 (~0.32 mg kg⁻¹: mg kg⁻¹), significantly lower than the soil. Research is yet to confirm whether the unusual accumulation of Ca in the foliar tissues of *P. rufuschaneyi* has an effect on the Ni tolerance of this species or counterbalances the soil Mg:Ca imbalance. Other Ni hyperaccumulators, e.g. *Odontarrhena chalcidica* (syn. *Alyssum murale*), show the same counterbalance of the magnesian character of the soil (Bani et al. 2007).

In addition to weathering, leaf litter and litterfall, throughfall, stemflow and atmospheric deposition may also contribute to the biogeochemical cycling of elements in an ultramafic system. This study reveals substantial contribution of throughfall to Ni flux, as high as 22%, whereas stemflow and atmospheric deposition have no significant contribution, <1% (Table 4; Figure 14). This flux could be explained partly by leaching of Ni from senescent leaves that are more concentrated. In the case of P, S and K, the throughfall contributes significantly to the respective fluxes, with 2-, 220- and 20-fold higher compared to the contribution by litterfall, respectively (Table 4). The atmospheric deposition of S is relatively higher (17 g m⁻² y⁻¹) compared to K and P and could explain partly high S concentration in throughfall.

This study reveals that the occurrence of saplings of *P. rufuschaneyi* is negatively correlated with the number of matured *P. rufuschaneyi* stand (Figure 12). This observation is inconsistent with the elemental allelopathy hypothesis, which posits that saplings of hyperaccumulators may have a competitive advantage over that of non-hyperaccumulators growing in a highly Ni enriched topsoils due to Ni recycling from high Ni litter (Boyd and Martens 1998; Boyd and Jaffré 2001). This hypothesis suggests that saplings of Ni hyperaccumulators may prefer to grow under matured Ni hyperaccumulators where Ni

availability is high due to leaf litter Ni enrichment. This is not the case in our study possibly because *P. rufuschaneyi* is well adapted to open areas (van der Ent et al. 2015b; Bouman et al. 2018). Hence, the shade from the canopy of the mature plants may not be suitable for the optimum growth of the saplings.

This study provides insights that have major implications for economic Ni agromining in the tropics. High Ni phytoavailability is a desirable trait for sustainable Ni agromining (Nkrumah et al. 2016; 2018a). Agromining operations harvest Ni-rich shoot biomass, which significantly limits Ni recycling through leaf litter. This implies that the labile Ni pools may deplete over time, indicating agromining operations are finite. Nevertheless, subsoil may replenish the topsoil labile pools (Chaney et al. unpublished), and non-labile pools may also contribute but this can be a slow process and depends on the local buffering capacity (Massoura et al. 2004). The number of cropping years required to deplete the labile Ni pools is unknown. The removal of biomass during agromining also affects litter recycling of essential nutrients (P, S, K and Ca). Therefore, comprehensive agronomic systems, including inorganic fertilization (especially P) may need to be considered in a sustainable tropical agromining. The inherent ability of *P. rufuschaneyi* to efficiently uptake and accumulate K into foliar tissues suggests K fertilization may not be important, as this may possibly interfere with plant physiology which may eventually inhibit optimum plant growth and Ni uptake. Moreover, K is a potential contaminant in the eventual bio-ore (Vaughan et al. 2017). However, continual K uptake by *P. rufuschaneyi* from the K-impoverished ultramafic soils during agromining operations may warrant minor incorporation of K fertilization to avoid total depletion. Calcium addition may follow similar approach as K, but Mg fertilization may not be important because of the concomitantly high Mg concentrations in the local ultramafic soils. Throughfall contributes substantially to the S flux and must be taken into consideration during S fertilization. The saplings of *P. rufuschaneyi* prefer open areas, and this would influence the farming strategy, as demonstrated by Nkrumah et al. (2019a).

This study highlights the biogeochemical cycling of Ni and physiologically relevant elements (P, S, K, Ca) in a typical Ni hyperaccumulator dominated forest in a wet tropical ultramafic ecosystem. Future Ni isotopic fractionation investigations may further clarify the mechanisms underlying the efficient Ni uptake by the roots of *P. rufuschaneyi* and the origin of the Ni absorbed (issued from mineral weathering or from the already existing soil available pool); subsequent Ni translocation to mature leaves; phloem Ni redistribution to developing

tissues; Ni enrichment in topsoil by leaf litter Ni recycling; and role of weathering in replenishing labile Ni pools. Furthermore, molecular studies may unravel the transporters responsible for the high Ni uptake and accumulation by *P. rufuschaneyi*. Research is also required to ascertain the ecophysiological roles of Mg, P, S, K, Ca in *P. rufuschaneyi*.

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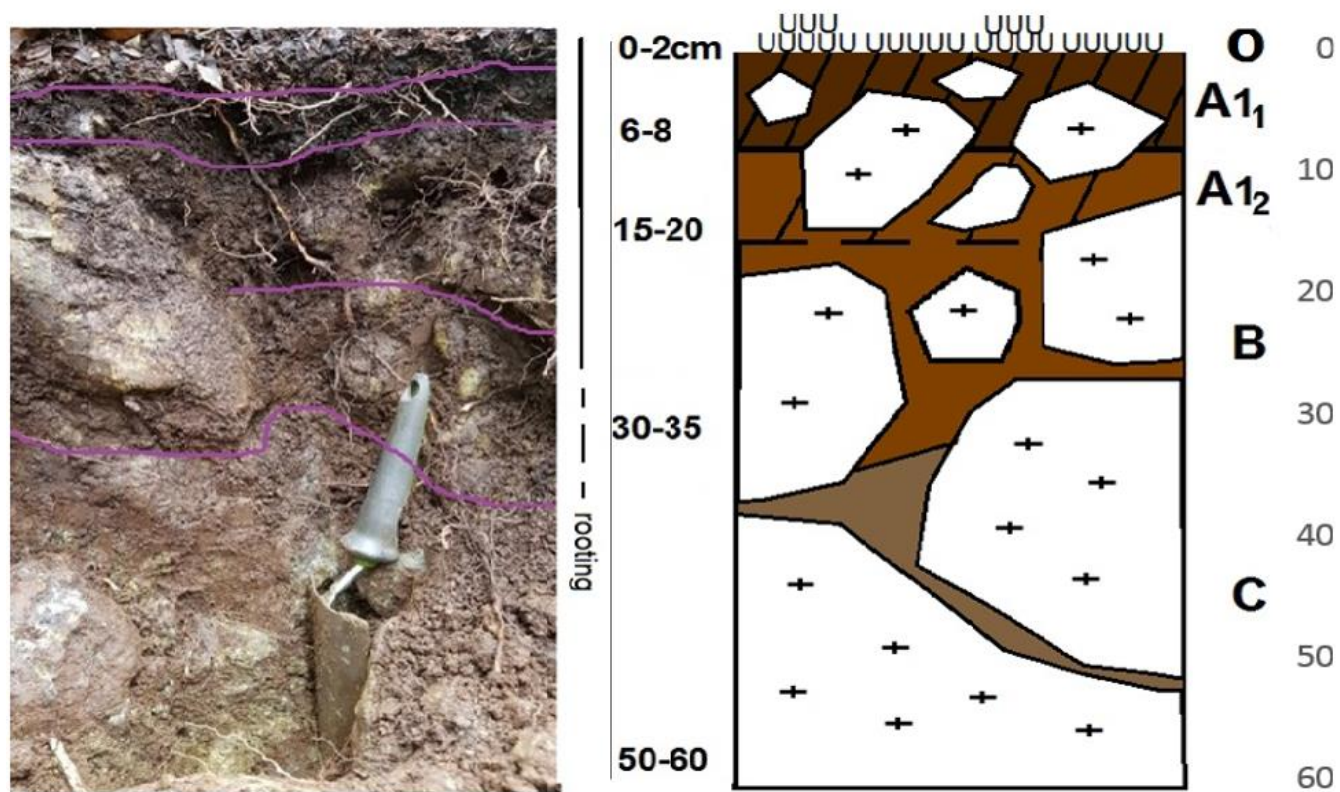
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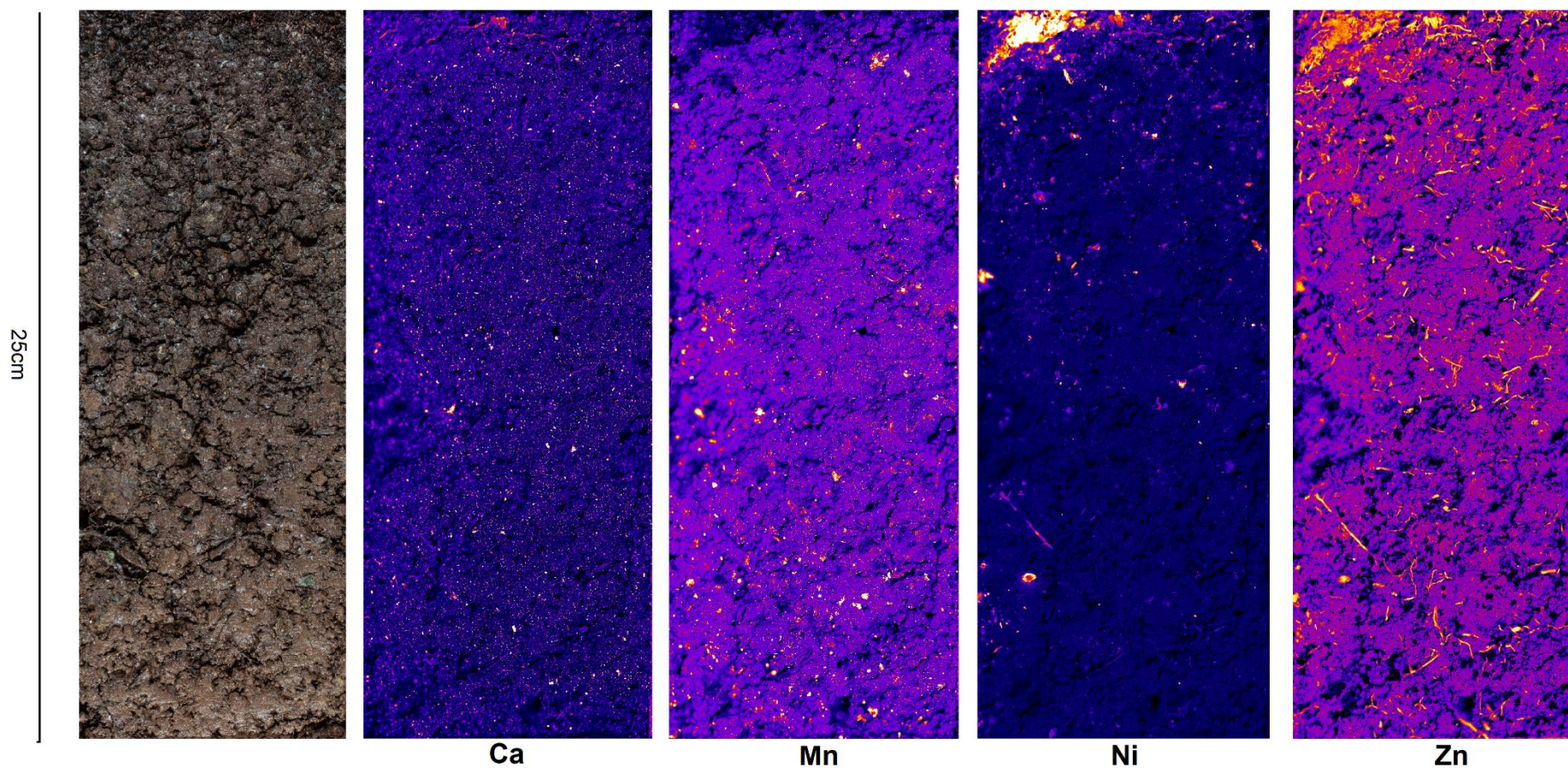
Supplementary Table 1. Average elemental concentrations in the rainfall, throughfall and stemflow from March 2018 to January 2020 (mg L⁻¹). Maximum, minimum and average elemental concentration in leaf litter of *P. rufuschaneyi* from March 2018 to January 2020 (g kg⁻¹).

			METALS							NUTRIENTS							
			Al	Co	Cr	Cu	Fe	Mn	Ni	Zn	Ca	K	Mg	Na	P	S	
Concentrations	mg L ⁻¹	Rainfall	--	--	--	--	--	0.01	0.03	0.06	0.6	0.3	0.03	0.3	0.05	13.8	
		Throughfall	Average	--	--	--	--	--	0.01	0.6	0.02	1.2	3.2	0.8	0.7	0.07	22.1
		Stemflow	--	--	--	--	--	--	0.4	0.01	1.2	3.0	0.5	0.8	0.3	19.5	
	g kg ⁻¹	Leaf litter (<i>Phyllanthus</i>)	Max	--	0.050	--	--	--	0.150	29.7	0.23	12.2	14.7	5.4	--	0.66	0.86
			Min	--	0.015	--	--	--	0.30	13.8	0.11	4.9	0.43	1.3	--	0.15	0.15
			Average	--	0.030	--	--	--	0.80	23.1	0.18	9.0	2.40	2.6	--	0.29	0.46

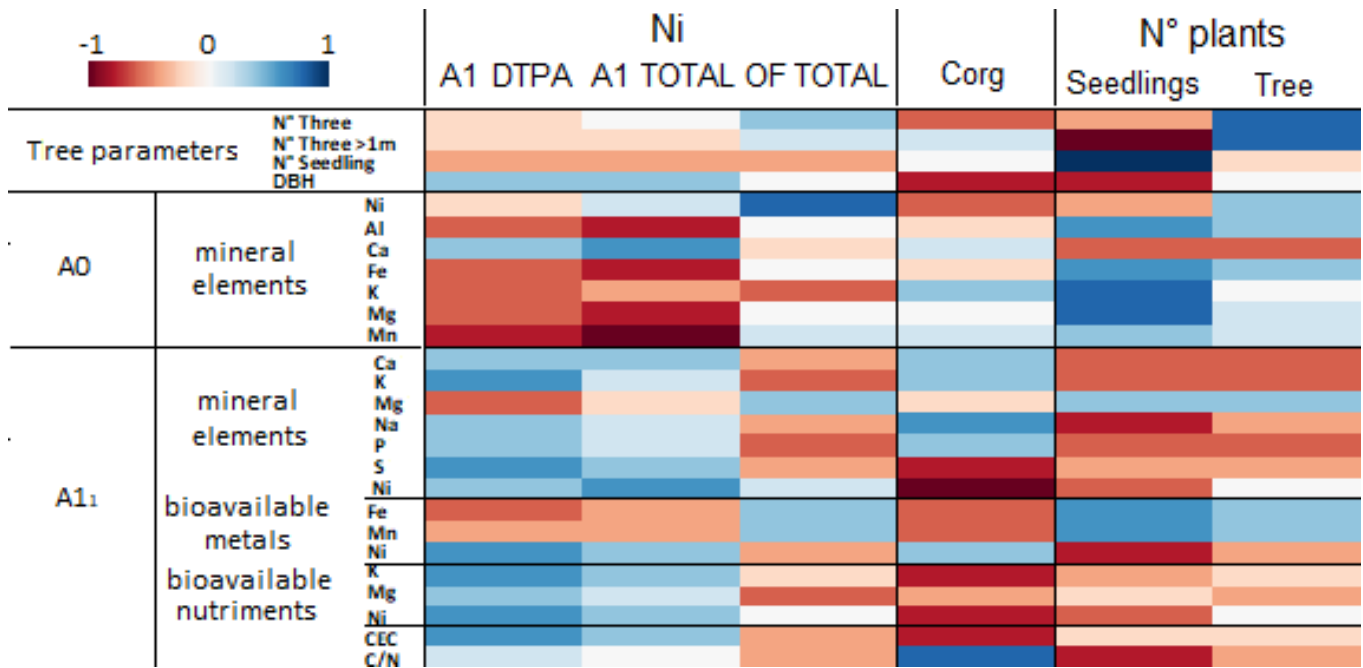
Supplementary Figure 1. Soil profile of the destructive plot of Garras' geochemical station



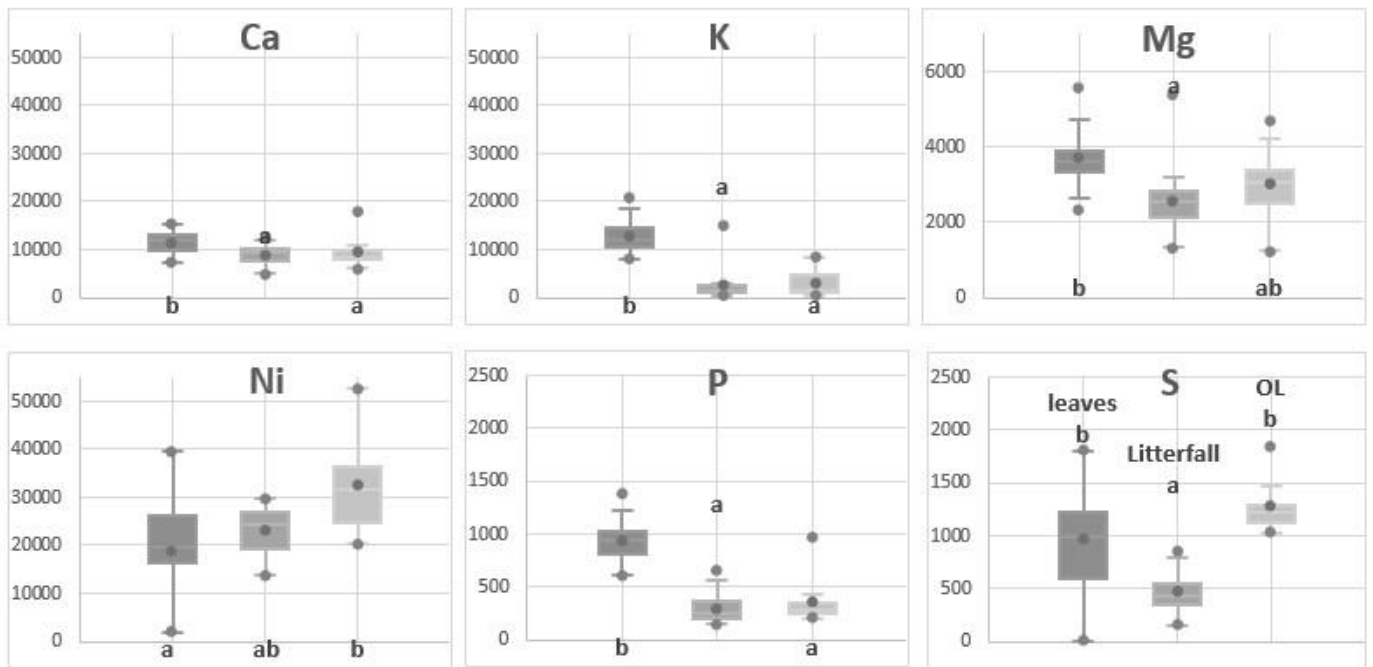
Supplementary Figure 2. Micro-X-ray fluorescence elemental maps showing Ca Mn Ni and Zn localisation on destructive plot's soil core



Supplementary Figure 3. Heatmap of correlation of element concentrations in soil and agronomic parameter



Supplementary Figure 4 Elemental concentrations (mg kg^{-1}) in leaves, litterfall and litter of the studied plot in November 2018.



CHAPTER 3: Element budget of a Tropical Metal Farm

Nickel stocks and fluxes in a tropical agromining ‘metal crop’ farming system in Sabah (Malaysia)

Romane Tisserand¹, Antony van der Ent², Philip Nti Nkrumah², Serge Didier³, Sukaibin Sumail⁴, Guillaume Echevarria^{1,2}

¹ Université de Lorraine, INRAE, Laboratoire Sols et Environnement, 54000 Nancy, France.

² Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, Queensland, Australia.

³ INRAE, Centre de Nancy, Ecosystèmes Forestiers, 54280 Champenoux, France

⁴ Sabah Parks, Kota Kinabalu, Sabah, Malaysia.

*corresponding author: Guillaume Echevarria

ABSTRACT

Nickel (Ni) hyperaccumulator plants play a major role in Ni recycling in ultramafic ecosystems, which may be more pronounced in a wet tropical environment. Nickel agromining operations which cultivate selected Ni hyperaccumulator plants (‘metal crops’) on Ni enriched substrates, including ultramafic soils, to extract valuable products could potentially affect the Ni dynamics in these ecosystems. In this study, we investigate the biogeochemical cycling of Ni (and nutrients) under agromining scenarios. *Phyllanthus rufuschaneyi* was studied in the first tropical metal farm located in Borneo (Sabah, Malaysia). For two years, we monitored nine plots of 25-m² and collected information on weather, biomass exportation, water and litter fluxes to the soil. Manipulation of the ecosystem to test the sensitivity of the ecosystem to the return of litter to the soil have been realised. Without harvesting, Ni inputs and outputs have minor contributions (< 1%) to the total Ni budget in a tropical hyperaccumulator forest ecosystem. The Ni cycle is mainly driven by internal fluxes, particularly litterfall and throughfall. After two years of cropping, the Ni litter flux corresponded to 50% of the total Ni stock in the aerial biomass (3.1 g m⁻² year⁻¹). Nickel is slowly released from the litter; after 15 months of degradation, 60% of the initial biomass and the initial Ni quantities were still present. Calcium, P and K budget are negative without fertilisation, sustainable agromining would lead to a depletion of Ca stock. Removal of P-, K- and Ca-rich biomass will eventually affect Ni yield, and these insights should be taken into account when designing sustainable tropical agromining. Weathering could replenish this stock on available nutrient on a long term and need to be further investigated.

Keywords: biogeochemistry; litter; nickel hyperaccumulator plants, nutrient cycling, Southeast Asia; ultramafic soils

A. INTRODUCTION

Hyperaccumulator plants have developed ecophysiological mechanisms to accumulate trace elements to extremely higher concentrations in their shoots compared to plants growing on the same substrates (Jaffré et al. 1976; Reeves 2003; van der Ent et al. 2013a). More than 700 hyperaccumulator species are currently reported, of which > 500 can hyperaccumulate nickel (Ni) (Reeves et al. 2017). To be classified as a Ni hyperaccumulator, a plant species needs to accumulate more than 0.1 wt% of Ni in its aerial biomass (Baker and Brooks 1989; van der Ent et al. 2013a). Nickel hyperaccumulators are restricted to ultramafic soils; these soils have particularly low concentrations of essential nutrients (such as P and K) but relatively high concentrations of trace elements (Cr, Co, Mn and Ni) (Proctor 2003; Echevarria 2018). Moreover, ultramafic soils have relatively higher Mg:Ca ratio compared to other soils (Proctor 2003).

Tropical regions represent a major global hotspot for hyperaccumulator plants, which are almost exclusively trees or shrubs. In the neotropics, Ni hyperaccumulators have been discovered in several regions for decades, including Brazil and Cuba (Reeves et al. 1996). In southern Mexico and Central America, at least 13 Ni hyperaccumulator have been recently discovered, including *Blepharidium guatemalense* and *Orthion subsessile* (McCartha et al. 2019; Navarette et al. 2021). In South Asia, the state of Sabah (Borneo Island, Malaysia) is fascinating for exploration because this island has extensive ultramafic soils and is one of the top five biodiverse regions in the world (Barthlott et al. 2017). In North Borneo, 32 plants are known currently to hyperaccumulate Ni (van der Ent et al. 2019); they are mainly from the Malpighiales order and Phyllanthaceae family (van der Ent et al. 2015b; Galey et al. 2017). *Phyllanthus rufuschaneyi* is a newly described species endemic to Borneo Island that can accumulate 2.5 wt% Ni in its leaves (Bouman et al. 2018), while in the roots and stems, Ni is mainly concentrated in the phloem (van der Ent et al. 2017).

Hyperaccumulator plants are increasingly being studied for their potential use in Ni recovery from the soil. Agromining, is a chain of processes where selected hyperaccumulator plants are cropped and harvested to recover a high-grade bio-ore (Barbaroux et al. 2011; Chaney et al. 2007; Nkrumah et al. 2018a). To be used for agromining, selected hyperaccumulators need to have a high biomass production and an ability to accumulate more than 1 wt% Ni in their aerial biomass ('metal crops') (Chaney et al. 2007; Nkrumah et al. 2016a, 2018b). Agromining has numerous advantages compared to traditional mining activities, such as improving fertility and protecting biodiversity (van der Ent et al. 2015). Agromining could rehabilitate mining soils and provide an alternative for agriculture on

degraded soil, which has the potential to significantly improve the livelihood of local farmers (van der Ent et al. 2015; Erskine et al. 2018). There are significant untapped opportunities for the economic exploitation of Ni agromining in the tropics (Nkrumah et al. 2016b, 2019d, 2021a). To date, five potential ‘metal crops’ have been discovered in Sabah, including *Actephila alanbakeri*, *Antidesma montis-silam*, *Dichapetalum gelonioides*, *Rinorea cf. bengalensis* and *P. rufuschaneyi* (Bouman et al. 2018; Nkrumah et al. 2018c, d; van der Ent and Mulligan 2015; van der Ent et al. 2016a).

Agronomic systems play significant role in developing sustainable Ni agromining (Bani et al. 2015; Li et al. 2013; Nkrumah et al. 2018b, 2021b). The agronomic systems for Ni agromining in a wet tropical climate have been recently undertaken in a pot trial employing two ‘metal crops’ (*P. rufuschaneyi* and *R. cf. bengalensis*) in Sabah (Nkrumah et al. 2019a, b, c). Organic matter and potassium (K) treatments seem to reduce Ni yield of *P. rufuschaneyi*, whereas sulphur (S) and phosphorus (P) increase Ni yield (Nkrumah et al. 2019a, b, c). Moreover, the first tropical agromining farm has been set up in 2016 in Pahu (Sabah, Malaysia) (Nkrumah et al. 2019d) to provide real life evidence of tropical Ni agromining, and a model for further studies. In the field trial, Ni yield was estimated to reach 250 kg ha⁻¹ using a plant density of four plants per m² (Nkrumah et al. 2019d). However, a Ni yield of only 75 kg ha⁻¹ was obtained in the field employing two plants per m² (See chapter 4). There are significant knowledge gaps that require investigations to optimise tropical Ni agromining in the field (Nkrumah et al. 2021a). The threshold of available Ni concentrations required for optimum plant uptake and shoot Ni accumulation is unknown. The number of cropping years required to deplete the available Ni pool is presently unknown. The role of subsoil Ni in replenishing the available Ni pools in the topsoil needs investigation. Moreover, the effect of harvesting Ni rich biomass on the available Ni pool (and essential nutrients) in the topsoil is unknown. The influence of climatic conditions (e.g. precipitation) on the cycling of Ni (and essential nutrients) in a tropical Ni agromining is yet to be determined. Detailed studies on Ni (and other elements) biogeochemical cycles could help address some of these knowledge gaps and contribute to the development of robust agronomic systems for sustainable tropical Ni agromining.

Ultramafic soils are the highest sources of Ni in the terrestrial systems (Estrade et al. 2015). Nickel and other metal mobility and accumulation can be influenced by different parameters (Kalbitz and Wennrich 1998) such as organic matter. Previous studies have investigated the local soil chemistry and foliar elemental concentrations in tropical hyperaccumulator plants (van der Ent and Mulligan 2015; van der Ent et al. 2015b; 2016b, 2018), but hyperaccumulator litter degradation has received little attention. For the temperate Ni ‘metal crop’ *Odontarrhena chalcidica*, Echevarria (2021) found

that leaf Ni leaches rapidly after decaying. Boyd et al. (2008) showed that the degradation of herbaceous hyperaccumulators can be fast; after one month, only 9–28% of original Ni content remained. Hyperaccumulator plants are responsible for a significant part of the available Ni in topsoil due to Ni rich leaf litter recycling (Estrade et al. 2015; Echevarria 2018; Ratié 2019; Paul et al. 2021; Zelano et al. 2020). Large quantities of lighter Ni isotope having biotic origin Ni are found in the upper soil layer of hyperaccumulator forest (Zelano et al. 2020). Sustained harvesting could deplete quantities of Ni available; nevertheless, this process could also promote the transfer of non-labile soil Ni to phytoavailable forms.

This study aims to assess the fluxes of Ni and other elements in a tropical metal crop system at the Pahu Agromining Trial Farm in Sabah, Malaysia. To that end, the concentrations and annual fluxes of Na, Mg, Al, P, S, K, Ca, Mn, Fe and Ni were measured in trial plots in the metal farm. Total and available stock of these elements were also studied.

B. MATERIALS AND METHODS

a. Site and plant study

This study was undertaken at the Pahu Agromining Trial Farm (Nkrumah et al. 2018; Tisserand et al. 2020), close to Pahu village, North Borneo, Sabah, Malaysia (335m asl; 6°7'49"N;116°46'22"E). This ultramafic Eutric Chromic Cambisol have already been described (Nkrumah et al. 2019a, Tisserand et al. 2021). *Phyllanthus rufuschaneyi* is one of the Ni hyperaccumulator studied in this farm due to its highly desirable traits for agromining, including global high shoot Ni concentrations with up to 3.2 wt% in old leaves, rapid re-growth after coppicing, tolerance for exposed conditions and pest resistance (Bouman et al. 2018; van der Ent et al. 2015b; van der Ent and Mulligan 2015; Nkrumah et al. 2019a, Tisserand et al. 2021). For this experiment, *P. rufuschaneyi* saplings were collected in their native habitat and transplanted into polyethylene bags containing ultramafic soil in a shade house. The soil used was homogeneous topsoil from the Pahu ultramafic field (see Nkrumah et al. 2019a; Tisserand et al. 2021). Transplantation into the field plots occurred on February 2018 with plants of 25 (\pm 8) cm height; nine 25 m² plots were planted at a density of two shrubs per square meter.

Treatments

In November 2018, an initial homogeneous NPK fertilisation (370 kg ha^{-1}) were applied to avoid nutrient limitation before the application of the organic treatment. From November 2018, hyperaccumulator plants were amended with litter four times with three replicates. A control treatment was put in place. Every four months, two different rates of litter: 250 or 500 g m^{-2} were applied, until a complete treatment application of 1 kg m^{-2} for the lowest dose and 2 kg m^{-2} for the highest dose in were achieved in November 2019 (Figure 16). These litter treatments consisted of *P. rufuschaneyi* shoot biomass crushed with a shredder and composed of 1 wt\% Ni , 0.8 wt\% K , 0.7 wt\% Ca and 0.6 wt\% P (Tisserand et al. 2021). Litter bags, with small mesh ($20 \times 20 \text{ cm}$; 1 mm mesh), were laid on the soil surface in all plots at each treatment application to be able to follow the litter degradation. The small mesh size of the nylon bags prevented loss of small leaf fragments and allowed access by bacteria, fungi and small soil invertebrates.

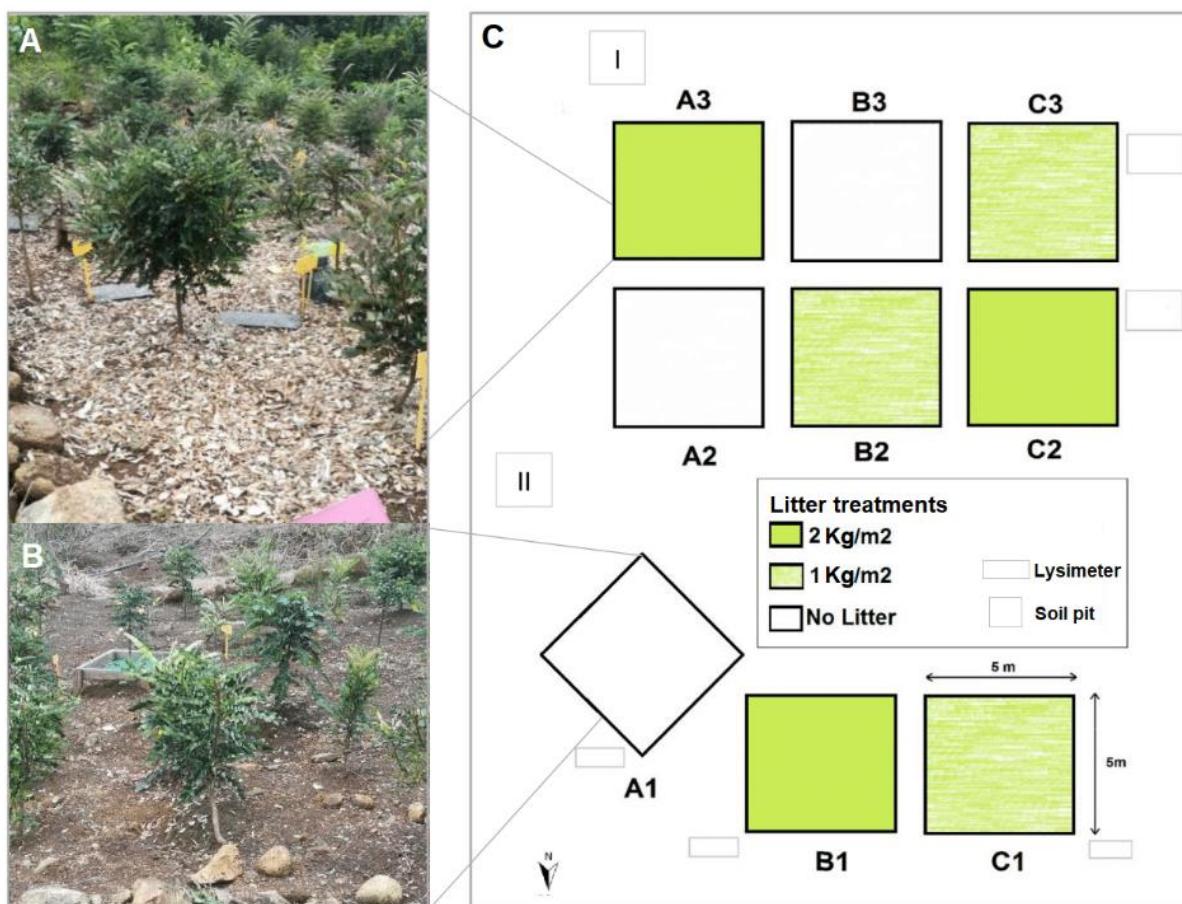


Figure 16 Overview of the biogeochemical plot of the first tropical metal farm in Sabah, Malaysia. A: *Phyllanthus rufuschaneyi* amended with litter; B: *Phyllanthus rufuschaneyi* non-amended with litter; C: Localisation of the litter treatments.

Biogeochemical station

In March 2018, a biogeochemical station was installed in the plot (Figure S5). A weather station, gutters, stemflow collectors, lysimeter and litter traps were randomly positioned at each subplot to monitor the following fluxes. Rainfall, throughfall, and stemflow samples were collected every two weeks at a minimum, and gravity water samples were collected every month at a minimum. Gravity water samples were collected before each treatment at 15 cm and 30 cm depth. Nine litter collectors with a surface area of 0.25-m² were installed 10 cm above ground level. Agronomic parameters measurement and litter collection were scheduled every month (diameter, high, branches numbers).

Soil collection and analyses

Soil was collected eight months after planting; four soil cores were collected in each subplot before treatment (depth: 0–5 cm, 5–10 cm, 10–15 cm). Two pits were dug transitional horizon (± 60 cm), one at the southern and the other at the eastern part of the plot in January 2020 to collect soil samples along the soil profile (Figure 16); the profile was divided according to the number of visible horizons (top-soil: A₁ and A₂, accumulation horizon: B and degradation horizon: BC). The soil pH was measured after solid suspension of dry soil sieved at 2 mm with deionised water at a ratio of 1/5 (m/v) (NF ISO 10390). Carbon and nitrogen content were quantified by combustion at 900 °C with a CHNS analyser (vario MICRO cube, Elementar Analysensysteme GmbH). Bioavailable nutrients and metals were extracted using hexaamminecobalt(III) trichloride (ISO/DIS 23470) and diethylene triamine pentaacetic acid (DTPA) (NF ISO 31- 121), respectively. The soil pseudo-total concentrations were determined by digesting 0.5 g of each soil sample with 2 ml HNO₃ and 6 ml HCl for 2 hours at 95 °C before filtration and analyses with ICP-AES (Thermo Fisher iCAP 6300 Duo). For all the protocols, blanks and standard reference materials were analysed.

Biomass measurement and litter analyses

One year after the initial treatment application, 12 plants per treatment were collected in January 2020. Plants were separated into different fractions after washing the roots with ultrapure water (roots, stems and leaves). Samples were dried for 48 hrs at 40 °C prior to grinding. Pseudo total element concentrations were determined in plant samples after digestion for 16 hrs with 1 mL HNO₃ (70%) and 2 mL H₂O₂ (30%) at room temperature after which the samples were heated for 2 hrs at 95 °C in a DigiPREP® system for 120 min at 95 °C prior to filtration 0.45 µm (Millipore). Each solution was analysed with ICP-OES (Thermo Fisher iCAP 6300 Duo). The recovery rates for Ni varied between 90–110%. Tree litter bags were collected from the treated plots at 1, 2, 4, 8 and 15 months. After

collection, each bag was dried and separated (leaves, petioles, wood and dust) and then weighed. Litter bags samples were analysed with the same protocols for plant samples presented in the previous paragraph. To measure soil contamination, composite samples of each plant organ were heated in muffle furnace at 550 °C for three hours to remove entirely organic matter. After cooling to room temperature overnight, the samples were reweighed to calculate mass loss.

Water measurement and analyses

After collection, water samples were filtered at 0.45 µm and were then stored refrigerated (~4 °C) until analysis. Electrical conductivity and pH were measured for each date. Total organic and inorganic carbon were analysed by infrared absorption of the CO₂ released after carbon combustion using a Shimadzu TOC 5050 instrument, while sulphate was measured using ion chromatography (940 Professional IC Vario). Samples for total element analyses were acidified (0.5%) with ultrapure HNO₃ before analysis with ICP-AES (Thermo Fisher iCAP 6300 Duo).

Calculation of the elements flux and litter degradation

From March 2018 to January 2020 total rainfall (TR) was 3720 mm (mean of ~2180 mm per year) and a mean temperature of 24.9 ± 0.6 °C. Atmospheric deposition was calculated using the elemental concentrations in the rainfall. Stemflow; throughfall and litter fluxes were calculated considering the average density of two plants per square meter.

Total nutrient input (I_t in $\text{g m}^{-2} \text{y}^{-1}$) is calculated by adding the quantities of nutrients in litterfall (Lf), stemflow (Sf) and throughfall (Tf):

$$I_t = Sf + Lf + Tf + DD \quad [\text{g m}^{-2} \text{y}^{-1}] \quad (4)$$

The total nutrient output was calculated by adding the quantities of nutrients ($\text{g m}^{-2} \text{y}^{-1}$) in gravity water at 30 cm depth (GW) and in harvested biomass (HB):

$$O_t = GW + HB \quad [\text{g m}^{-2} \text{y}^{-1}] \quad (5)$$

The annual budget (AB in $\text{g m}^{-2} \text{y}^{-1}$) was calculated by subtracting nutrients output to nutrients inputs:

$$AB = I_t - O_t \quad [\text{g m}^{-2} \text{y}^{-1}] \quad (6)$$

For the litter degradation experiment, decomposition rate constant (k) was calculated by subtracting weight at the time t (W_t in g) from weight at the initial time (W_0 in g) and dividing by time t in y^{-1} (Olson 1963):

$$k = (W_0 - W_t) / t \quad (7)$$

The Ni percentage remaining in litter bags (Nirem in %) was determined by dividing Ni quantity at the time of collection (Nit in mg) by initial Ni quantity (Ni0 in mg):

$$\text{Nirem} = (\text{Nit} / \text{Ni0}) * 100 [\%] \quad (8)$$

Ni litter degradation flux is calculated by using the quantity of Ni applied per square meter (Nitreat in mg):

$$\text{NiLD} = (1 - (\text{Nirem} / 100)) * \text{Nitreat} [\text{Ni mg m}^{-2}] \quad (9)$$

To know the impact factor on biochemical cycles, translocation factor (TF) was measured by dividing elemental concentrations (mg kg⁻¹) in shoots by roots:

$$\text{TF} = \text{Cshoot} / \text{Croots} \quad (10)$$

Statistical Analysis Data

Statistical analyses were performed using R software, version 3.6.2 (2019-12-22). The significant difference between treatments was tested using Kruskal-Wallis test with a confidence level of 95%. Post-hoc pairwise comparisons were performed using Dunn's test.

C. RESULTS

a. Elements concentrations and stocks in soil

Table 6 Total, DTPA and CEC elemental concentrations in mg kg⁻¹ of the two pits of Pahu field in January 2020.

N°	TOTAL									DTPA		CEC			cmol ⁺ kg ⁻¹
	Ca	Cr	Fe	K	Mg	Ni	P	S	Zn	Ni	Ca	K	Mg		
A1 ₁	I	4800	7300	140 000	160	50 000	4100	250	470	110	110	2900	275	1500	24
	II	4500	6000	600	50 000	5100	100	3700	45	900					
A1 ₂	I	3400	11 000	190 000	60	55 000	4900	130	280	120	70	2500	-	800	19
	II	4000	7100	160 000	110	5100	180	410	110	130	2100	34	1500	25	
B	I	2200	6100	240 000	110	60 000	8700	100	250	110	90	2100	-	2200	31
	II	2300	4300	150 000	40	6400	70	210	80	100	1000	-	3600	37	
BC	I	1200	4700	190 000	40	62 000	6600	-	120	80	94	550	-	4000	39
	II	3200	150 000	30	70 000	5900	90	90	330	-	4600	44			

Nickel concentration varied along soil profile (Table 6). For the first pit, Ni concentration was lower in the A1₁(4100 mg kg⁻¹) than A1₂ horizon (4900 mg kg⁻¹), whereas in the second pit, Ni concentration was constant between these two horizons (5100 mg kg⁻¹). Although the pseudo-total Ni concentration of the latter was constant, its DTPA-extractable Ni concentrations increased from 110 mg kg⁻¹ in A1₁ to 130 mg kg⁻¹ in A1₂. For the first pit, DTPA-extractable Ni decreased from 110 mg

kg⁻¹ in A₁ to 70 mg kg⁻¹ in A₁₂. B horizon had the highest Ni concentrations in both pits (Table 6). The Mg concentration did not vary between the two pits but increased from 50 g kg⁻¹ at the surface to 70 g kg⁻¹ at BC horizon. However, Ca concentrations had an opposite trend to Mg (Table 6). Potassium decreased with depth, from 160 to 40 mg kg⁻¹ in the first pit and 600 to 30 in the second pit (Table 6). In the spatial distribution, K varied strongly in the topsoil (160 and 600 mg kg⁻¹) compared to the BC horizon (40 and 30 mg kg⁻¹), in pits 1 and 2, respectively.

Table 7 Elemental stocks in soils surface and plants (roots; stems; leaves) in g m⁻² in January 2020. Only means followed by letter are significantly different ($p < 0.05$ level).

		Litter treatment kg m ⁻²	Cr	Mn	Ni	Zn	Ca	K	Mg	Na	P	S		
Concentration mg kg ⁻¹	Leaves	0	30	240	20 000	190	7000	6400	3300	--	880	2300		
		2					9600	7500	3100		940	2500		
	Stem	0	20	80	7400	80	6900	9400	1400	310	1700	1500		
		2	40	80	7800		10 000	10 000	440	2100	2900			
	Roots	0	150	120	11 300	120	5000	5300	3300	490	730	1400		
		2	330	210	10 600		6000	5500	4600	660	660			
	Soil	0-5cm	0	6400	4800 b	5400	110	2800	140 a	47 000	40 a	150	190 a	
			2		4200 a	6500		3500	390 b	43 000	150 b	280 b	280 b	
		10-15cm	0	6700	4500	5900		3600	90 a	55 000	40	80	180	
			2	6000	4400			3200	220 b	50 000	60	180	230	
		Leaves	0	--	0.07	5.4		0.05	1.9	1.6	0.8	--	0.2	0.6
			2		0.06	5.3			2.5	2.0	0.8		0.6	0.5
Stock g m ⁻²	Roots	0	0.02	0.02	2.0	0.02	0.8	1.2	0.4	0.05	0.2	0.3		
		2		3.0	1.9		0.7	0.5	0.2	0.2	0.3			
	Plant	0	0.02	0.08	8.7	0.02	3.8	3.6	1.2	0.10	0.7	0.9		
		2		0.09	9.7		6.2	5.2	1.3	0.25	0.9	1.3		
Soil	Total 0-15cm	0	2500	1800	2400	40	1300	110	18 000	40	90	90		
		2			2300		1400	60	21 000	20	50	80		
	DTPA 0-15cm	0		27	52	0.2								
		2		24	100									

The elemental concentrations in the surface soil (0–5 and 10–15 cm) of each plot are presented in Table 7; S2; S3; S4; S5. In the 0–5 cm soil depth, Ni increased (1.2-fold) in the 2 kg m⁻² litter treated plot compared to the control ($p = 0.08$) (Table 7). Phosphorus, S, K and Ca increased by 1.9-, 1.5-, 2.8- and 1.3-fold, respectively, compared to the control ($p < 0.05$). However, Mn concentrations were relatively higher in the control (4800 mg kg⁻¹) relative to the treated plot (4200 mg kg⁻¹) ($p = 0.04$). In the 10–15 cm soil depth, only K concentration was statistically higher in the treated plot compared to the control ($p = 0.03$). In January 2020, Ni available stock in the surface soil was 52 kg m⁻² for non-amended soil, and 100 kg m⁻² for litter treated soil; Ni stock in the surface soil was 2.4 kg m⁻² for non-amended soil, and 2.3 kg m⁻² for litter treated soil (Table 7). Notably, Ni is the third highest stock, after Cr (2.5 kg m⁻²) and Mg (1.8–2.1 kg m⁻²).

Elemental concentrations and stocks in plant

Nickel concentrations of *P. rufuschaneyi* varied significantly in the plant fractions ($p < 0.01$) (Figure 17; Tables S5, S6). The leaves had the highest Ni concentrations, reaching 3.2 and 3.4 wt% in old and young leaves, with mean concentrations of 2.2 and 1.9 wt%, respectively (Figure 17). The primary roots were relatively enriched in Ni compared to old stem (1.6-fold) ($p < 0.05$); the old stems had the lowest Ni concentrations relative to all plant parts (Figure 17). Calcium and K are relatively enriched in the leaves, with 0.7 wt% and 0.6 wt% in the control treatment and 1.0 wt% and 0.8 wt% in the litter treatment, respectively, compared to Mg (Table 7).

The leaves consisted of two-thirds of the aerial tree biomass. Almost two years after planting, the Ni stock in the *P. rufuschaneyi* in the control plot was 8.7 g m^{-2} , with 6.7 g m^{-2} in aerial biomass out of which 5.3 g m^{-2} was in the leaves (Table 7). For plants amended with litter, the Ni stock was slightly higher (9.7 g m^{-2}) but not statistically significant. Nickel had the highest stock in biomass relative to all the elements, followed by Ca and K (Table 7).

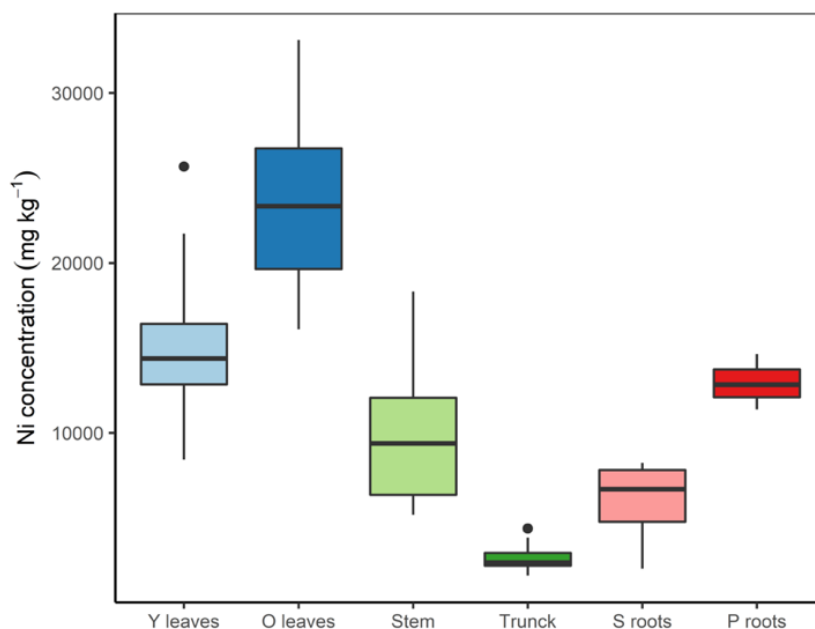


Figure 17 Nickel concentrations in the different parts of *Phyllanthus rufuschaneyi* in January 2020.

Water fluxes

Mean concentrations of ions in precipitation, throughfall, stemflow and gravity water are shown in Figure 18. Statistical variations between treatments were significant for some elements (Ca, K, Mn, Na, Ni and P) and some instruments. Drainage was higher for Ca and Na for the higher litter treatment, notably at 30 cm. Tendencies of Fe loss in the high dose treatment was visible ($p = 0.13$). Throughfall and stemflow concentrations were higher than lysimeter for K, Mn, Ni, P and Zn ($p < 0.01$).

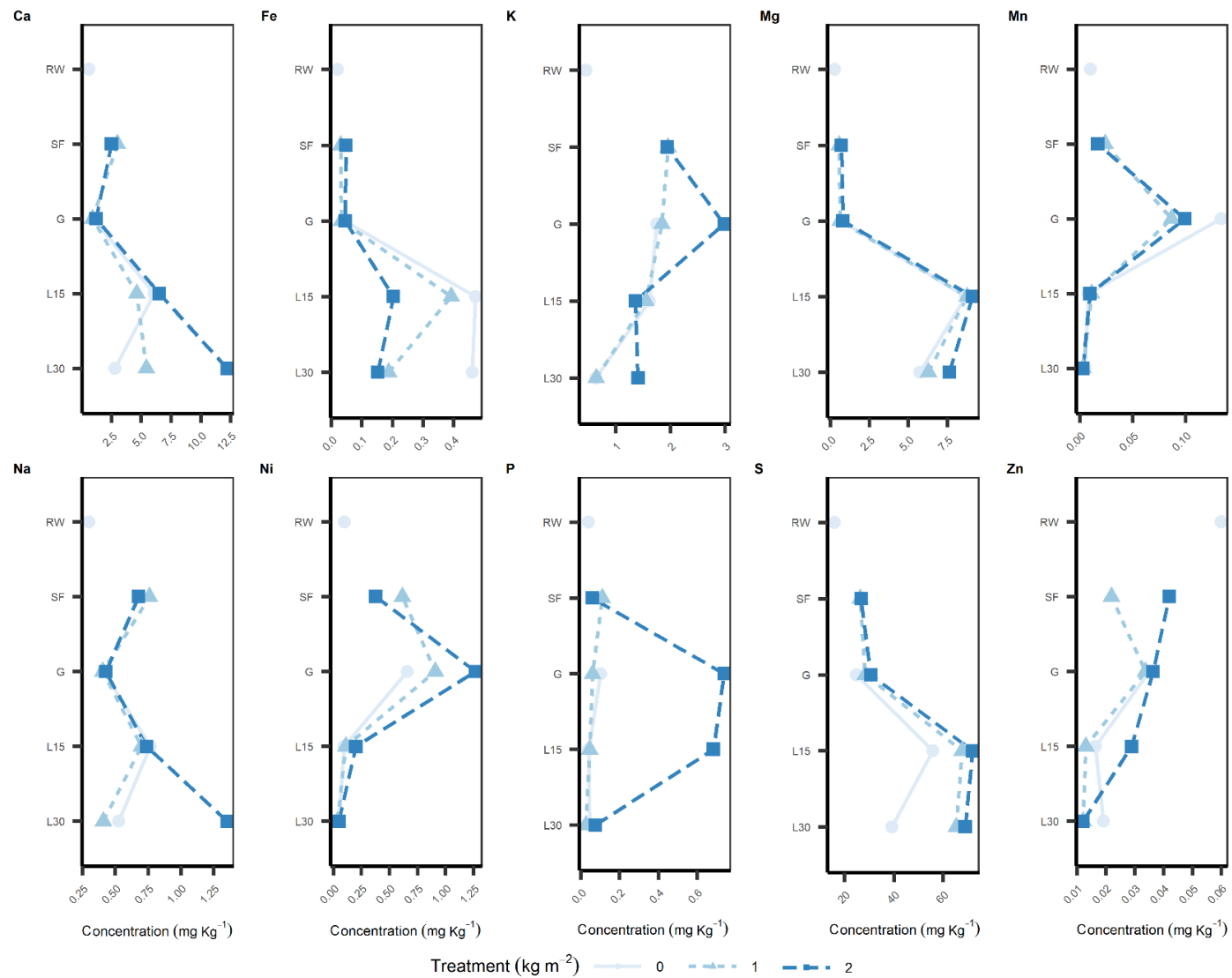


Figure 18 Element concentrations of water of the different litter treatments in mg kg⁻¹. RW: Rainwater; SF: Stemflow; G: Throughfall; L15: Gravity water 15

The concentrations of all ions studied, except Zn, were higher in throughfall and stemflow than in precipitation ($p < 0.01$). The dominant elements in throughfall and stemflow were S, Ca and Mg. Nickel concentrations were higher in rainfall and throughfall relative to drainage water and stemflow, respectively. Throughfall from plants amended with litter contained more K (3.0 mg L^{-1}) and Ni (1.3 mg L^{-1}) than plants non-amended with litter ($p < 0.01$). On the contrary, throughfall of control plants was more concentrated in Mn (0.13 mg L^{-1} ; $p < 0.01$). Stemflow of 2 kg m^{-2} litter treated plants was less concentrated in Ni (0.4 mg L^{-1}) and Mn (0.02 mg L^{-1}) compared to the 1 kg m^{-2} litter treated plants ($p < 0.03$). No other effect of treatments was visible on stemflow concentrations.

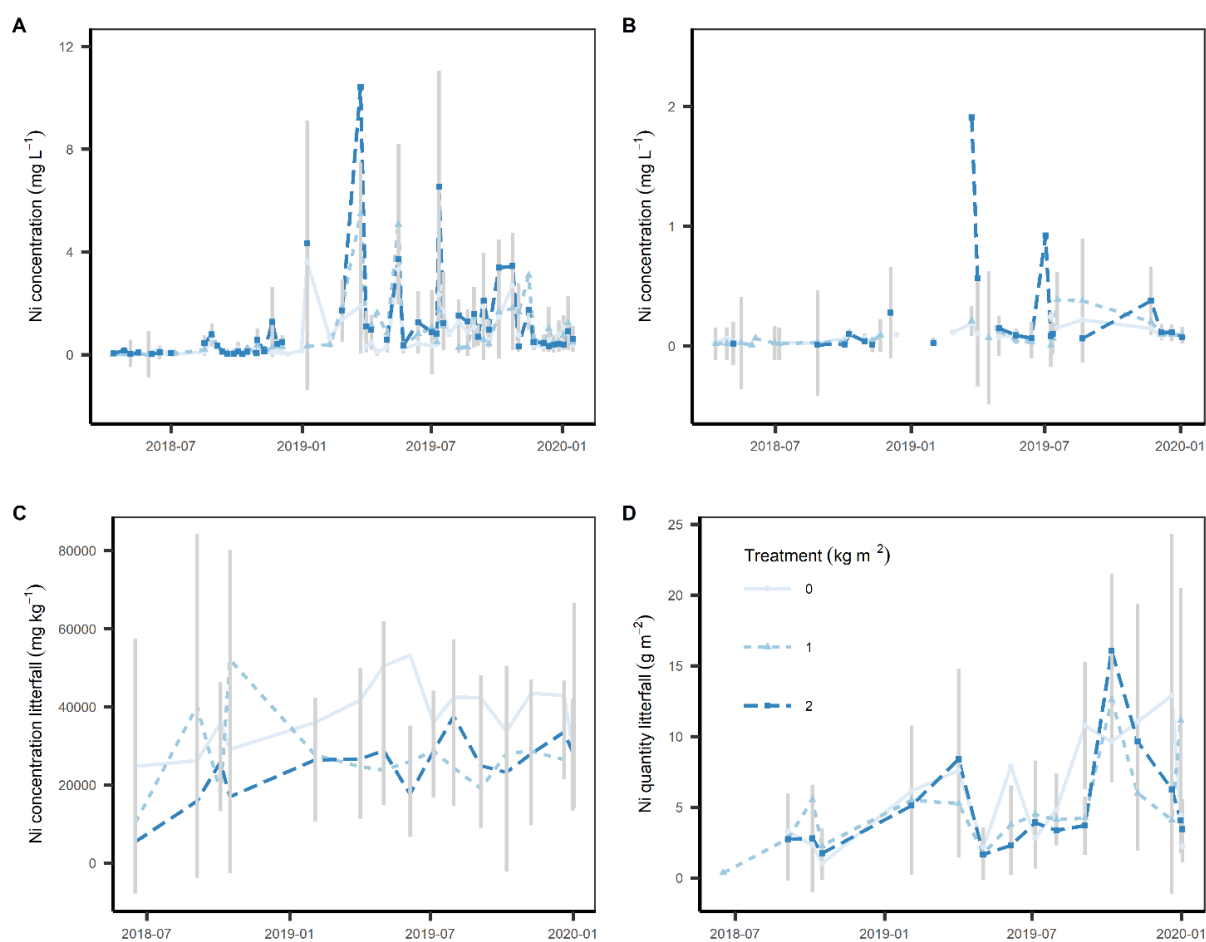


Figure 19 Ni concentration and quantity of Ni fluxes in function of time. A: Ni concentration of throughfall in mg L^{-1} ; B: Ni concentration of stemflow in mg L^{-1} ; C: Ni concentration of litterfall in mg kg^{-1} ; D: Ni quantity from litterfall in g m^{-2} .

The amount of precipitation, throughfall, stemflow and gravity water varied strongly with time. Driest season period was in February-March and wettest period was in December-January (Figure S6). Nickel concentrations in water varied with time from less than 0.05 mg L^{-1} at the beginning of the experiment to more than 10.4 mg L^{-1} in March 2019 (Figure 19). Nickel concentrations in drainage water were always lower than stemflow and throughfall. Nevertheless, Ni concentrations in gravity water tended to be higher at 15 cm depth for the high dose litter treatment ($p = 0.11$) (Figure 19). Until the end of 2018, Ni concentrations in the stemflow and throughfall were lower (Figure S7). Tendencies

of higher litter treatment to release more Ni in water is visible; Ni in throughfall is higher in the 2 kg m⁻² and occasionally higher in the 1 kg m⁻² treatment, notably in March 2019 compared to control (p <0.01). Ni quantities released from throughfall were two times higher for high dose treatment (2.2 g m⁻² y⁻¹) than non-treated plants (1.1 g m⁻² y⁻¹) (Figure 19). Throughfall is a major input flux for Mn, Ca, K, Mg, Na, P and S (Table 8). Stemflow has a relatively low effect on elements input compared to throughfall. Nevertheless, throughfall is overestimated because plant leaves do not cover all the soil surface.

Litterfall flux

Litter fluxes varied with time from July 2018 to January 2020 (Figure 19). Nickel concentrations were lower in June 2018 at the beginning of the experiment in litterfall and varied from 0.5 wt% to 5.3 wt%. Litterfall is less concentrated in Ni when plants were amended with litter (p <0.01). The quantity of Ni from litterfall was lower at the beginning of the experiment since plants had lower biomass stand; Ni quantity varied until 18 g m⁻² at the end of 2019. Litter flux was the major input flux for Ni and Zn. An input of 3.1 g m⁻² y⁻¹ of Ni by litter for non-treated plants was recorded in 2019. Litter flux was significantly lower for treated plants, about two time less (1.5–1.6 g m⁻² y⁻¹; p = 0.3) (Figure 19). Calcium input by litter (0.5 g m⁻² y⁻¹) was five times lower than Ni input by litter (Table 8).

Litter decomposition

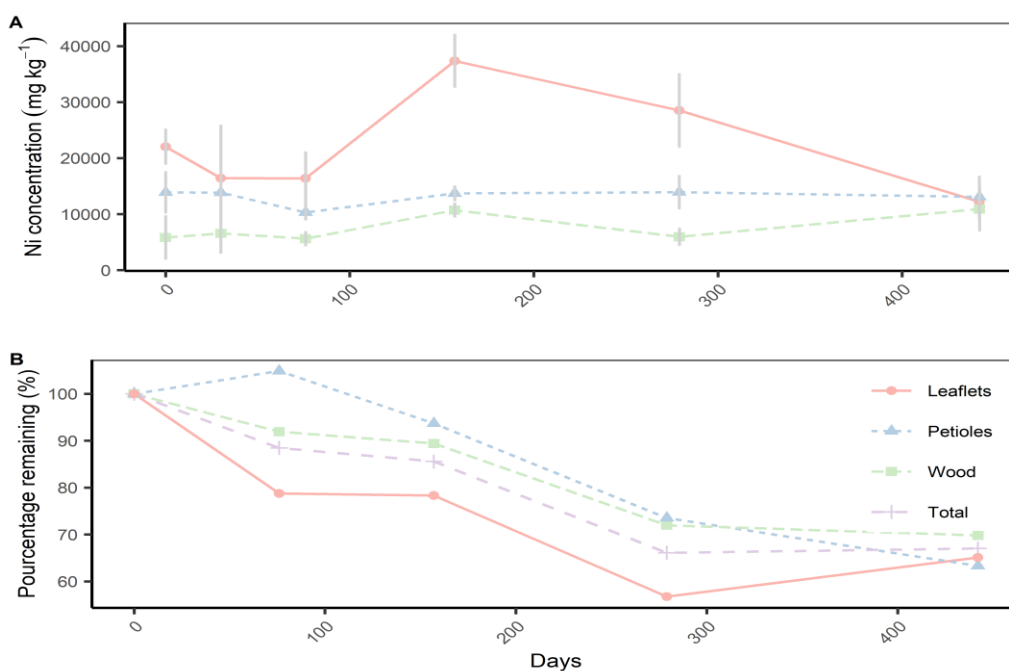


Figure 20 Litter bags biomass and Ni concentration in function of time from middle 11/2018 to end 01/2020 of the different component of the litter bags. A: Ni concentration of litterbags in mg kg⁻¹; B: Litter remaining in percentage.

Litter bags degradation reflected litter degradation of all the plots (Figure 20, Table S7). The constant of decomposition, k was 0.8 on average but varied with time and organs ($p < 0.01$). Leaflets degradation was faster ($k = 1.1$) than wood ($k = 0.3$) and petiole degradation ($k = 0.8$). More than 450 days after the first litter treatments application, 68% of the litter was remaining. Degradation varied as a function of the element studied. Litter decomposition rate was not a better indicator of Ni release from litter. During the first three months, Ni concentrations in leaflets and wood did not vary (2.2 wt % and 0.6 wt %). Ni concentrations in leaflets increased to 3.8 wt% after 4.5 months. Nickel concentration in leaflets was still 3 wt % eight months after the first litter input. Nickel concentrations in petioles did not statistically vary with time (1.3 wt%). Fifteen months after treatment application, Ni concentrations in all the plant fractions were similar (1 wt%). The release of K from litter was fastest relative to all element, completely leached out only after four-months (Table S7). Other elements such as Ca and S were not significantly leached out after 15 months.

Annual budget

Table 8 Elemental fluxes in $g\ m^{-2}\ y^{-1}$ from January 2019 to January 2020.

Litter	Quantity flux ($g\ m^{-2}\ y^{-1}$)	Mn	Ni	Zn	Ca	K	Mg	Na	P	S
0 $kg\ m^{-2}$	Dry Deposit	0.01	0.02	0.09	0.7	0.25	0.3	0.4	0.01	17
	Fertilisation*	--	--	--	--	5.4	--	--	5.4	--
	Throughfall	0.12	1.1	0.04	1.4	2.1	1	0.35	0.04	40
	Stemflow	--	0.004	--	0.02	0.01	0.005	0.003	0.001	0.2
	Litterfall	0.03	3.0	0.02	0.58	0.13	0.23	--	0.03	0.08
	Total nutrient return	0.16	4.1	0.15	2.7	7.9	1.5	0.75	5.5	57
	Drainage	--	0.01	--	0.25	0.11	1.2	0.06	--	4.3
	Harvesting	0.12	6.4	0.05	3.2	1.8	0.8	0.06	0.6	0.8
	Total nutrient loss	0.12	6.4	0.05	3.45	1.91	2	0.12	0.6	5.1
	Total soil annual budget	-0.11	-2.3	0.1	-0.75	6.0	-0.47	0.63	4.9	52
2 $kg\ m^{-2}$	Total nutrient return	0.12	3.8	0.20	3.1	8.4	1.4	0.74	5.5	69
	Total nutrient loss	0.12	6.4	0.05	3.75	1.85	1.3	0.11	0.6	6.1
	Total soil annual budget	--	-2.6	0.15	-0.68	6.6	0.12	0.63	4.9	63

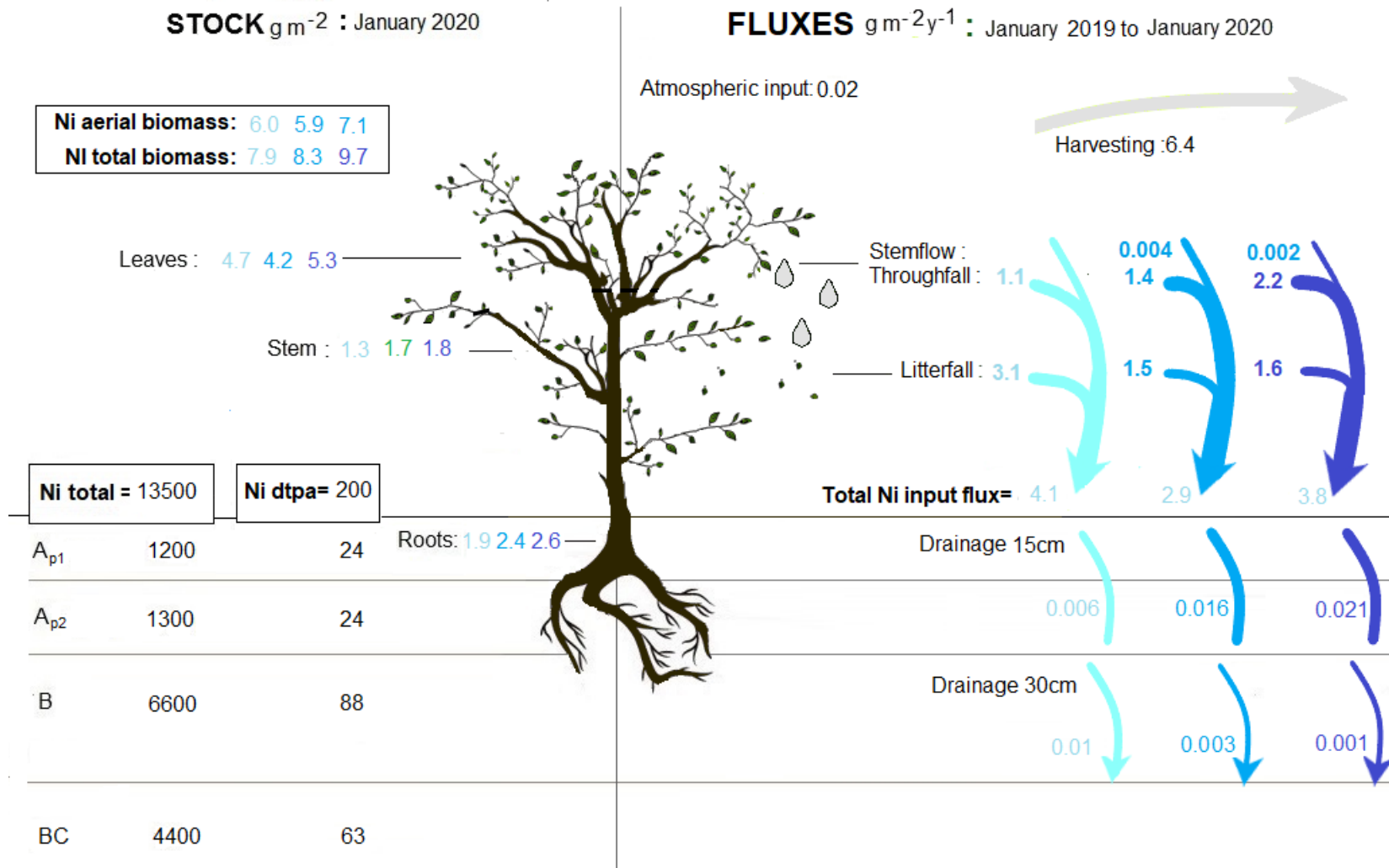


Figure 21 Nickel biogeochemical cycle of a *Phyllanthus rufuschaneyi* of the first tropical metal farm from January 2019 to January 2020. Stocks in g m^{-2} and fluxes in $\text{g m}^{-2}\text{y}^{-1}$. (Litter treatments kg m^{-2} : light blue = 0; blue=1; dark blue=2)

Annual element budget was calculated with flux from January 2019 to January 2020 (Table 8, Figure 21). This annual budget is positive for Na, P, S, K and Mn, negative for Mn, Ni, Zn and Ca. It is important to note that K and P budget are positive only due to the fertilisation applied in November 2018 and would be negative without it. The primary nutrient return was highest for S ($64 \text{ g m}^{-2} \text{ y}^{-1}$), followed by Ni ($4.2 \text{ g m}^{-2} \text{ y}^{-1}$) and Ca ($2.6 \text{ g m}^{-2} \text{ y}^{-1}$). Biomass harvesting was the main output for all the elements, except for S that was preferentially lixiviated ($4.1 \text{ g m}^{-2} \text{ y}^{-1}$). Nickel leaching was very low ($0.01 \text{ g m}^{-2} \text{ y}^{-1}$), even lower than atmospheric deposition ($0.02 \text{ g m}^{-2} \text{ y}^{-1}$). Nevertheless, Ni in gravity water is higher at 15cm than 30cm, so a part of the Ni leached in the surface horizon is reabsorbed by B horizon. Nickel cycle was mainly driven by litterfall ($3.1 \text{ g m}^{-2} \text{ y}^{-1}$) and biomass export ($6.4 \text{ g m}^{-2} \text{ y}^{-1}$ for two plants per m^{-2}). No statistical difference was visible between the annual nutrient budget of treated soil and control soil.

Biochemical sub-cycle

Only Mg accumulated preferentially in the roots, in contrast to all the other elements (Table 9). Translocation factors were influenced by litter for Mn and Mg (Table 9). Nickel translocation factor was 1.9. Leaves from plants amended and non-amended with litter had the same Ni ($20\,000 \text{ mg kg}^{-1}$) and Zn (190 mg kg^{-1}) concentrations. The same pattern was measured in the litterfall of these plants. Litterfall of control plants were more concentrated in Mn (420 mg kg^{-1}) and Mg (300 mg kg^{-1}) than litterfall of plants treated with litter. The latter were more concentrated in Ca (9800 mg kg^{-1}) and P (450 mg kg^{-1}) relative to the control. Concentrations between leaves and litter are different for numerous elements. Senescent leaves contained more Ni, up to 37% and 86% of the total Ni concentrations in whole plant in the treated and control plants, respectively. Calcium, Mn and Zn are also more concentrated in senescent leaves, while K, Mg, P and S are reabsorbed. Approximately 70%, 50% and 30% of P, S and K were reabsorbed by *P. rufuschaneyi*.

Table 9 Elemental concentrations in leaves and litter in kg m^{-2} , their differences in % and translocation factor (TF).

	Litter kg m^{-2}	Mn	Ni	Zn	Ca	K	Mg	P	S
Litter (mg kg^{-1})	0	420 a	38 000 a	320 a	8000 b	4400	3000 a	270 b	1200
	2	270 b	26 000 b	213 b	9800 a	5100	1900 b	450 a	1200
Differences (%)	0	91	86	77	45	-33	-3	-71	-48
	2	23	37	42	53	-30	-34	-54	-50
TF	0	1.8	1.9	1.5	1.1	1.2	0.9	1.3	1.6
	2	1	1.8	1.3	1.1	1.3	0.6	1.5	1.7

D. DISCUSSION

a. Balance and fluxes of Ni

Nickel concentration and availability in ultramafic soils vary as a function of soil chemical and mineralogical parameters (Echevarria 2021). This study revealed that Ni is principally stored in the BC horizon (6400–8700 mg kg⁻¹) of the plot, indicative of the substantial Ni concentrations in the subsoil. Moreover, the surface soils are enriched in Ni (4100–5100 mg kg⁻¹). The available Ni concentrations do not vary significantly along the soil profile, with DTPA-extractable Ni concentrations of 110 mg kg⁻¹ in the surface soils and 95 mg kg⁻¹ in the BC horizon (Table 6, S2). On the contrary, the topsoil of a typical Ni hyperaccumulator plants dominated forest is enriched in available Ni relative to the subsoil due to substantial Ni enriched leaf litter recycling (Tisserand et al. unpublished). The relatively high available Ni concentrations in the soil is indicative of the clayey nature of the ultramafic soil. Nickel extractability is relatively high in clayey ultramafic soils as well as soils dominated by amorphous Fe-oxides bearing phases compared to highly crystalline Fe-oxides (e.g. Geric Ferralsols) (Massoura et al. 2006; Raous et al. 2010; Echevarria 2018; van der Ent et al. 2018). Notably, the non-available Ni stock is also high and could potentially replenish the labile Ni pool, but this is a relatively slow process which depends on the local buffering system (Massoura et al. 2004). In two years, the total Ni concentrations increased, even without litter treatment (Table S3, 4, 5, 6). Plant cultivation could induce a release of Ni from weathering rocks. Nickel concentration in roots is significantly higher than DTPA-Ni, suggesting strong Ni uptake mechanisms. Nickel hyperaccumulator plants have been reported to take-up Ni from the same labile soil pool as non-hyperaccumulator plants, but at higher rates (Echevarria et al. 2006).

Total litter degradation has a constant degradation of 0.8 over a year on average, due to the high degradation of the leaf fraction (1.1) and the low degradation of the woody part (0.3). Boyd et al. (2008) and Echevarria (2021) showed that degradation of herbaceous hyperaccumulator plants could be relatively faster. In tropical climates, rapid litter degradation is expected due to high temperature and rainfall. However, the woody parts of tropical hyperaccumulator plants can slow the degradation process, as observed in this study. Nickel release from litter is not correlated with biomass degradation; Ni is immobilised after four months and is released slowly after 8 months of litter degradation. Nickel leached out from the litter increases the soil Ni available pool (Tisserand et al. 2021); Ni available stock was doubled in 15 months. However, the extent of availability of the Ni fraction is influenced by the prevailing local Ni bearing phases. Leached Ni from litter increases with amount of litter and tends to be higher in the A horizon relative to B horizon. Notably, the B horizon tends to be more

concentrated in clays that can store Ni and increase its stock. The roots of *P. rufuschaneyi* can develop to a depth of at least 40 cm (Tisserand, unpublished), and therefore, may access the Ni stock in the B horizon.

This study reveals that there is no significant increase in foliar Ni concentrations of *P. rufuschaneyi* growing on soil extremely rich in available Ni compared to the control plot, suggesting that there could be a minimum DTPA-extractable Ni concentrations threshold sufficient for maximum Ni accumulation in this species. The Ni stock in the hyperaccumulator biomass is mainly localised in both old and young leaves; concentrations in roots increase with litter treatment but the Ni stock in aerial biomass does not. Although litter treatment did not lead to an increase of Ni concentrations in living leaves, it increases Ni concentrations and quantities in throughfall. These observations could be due to an equilibrium between Ni intake in leaves and Ni losses through leaf leaching. Increase of Ni in throughfall could be also due to the higher number of aerial biomass (e.g. the leaf surface area). Litter from amended plants are 50% more concentrated in Ni than leaves and 86% more concentrated in Ni than litterfall from non-amended plants. No Ni depletion in plant organs of non-amended plants was visible, so we considered that this increase comes from a higher Ni return flux to balance the removal of Ni by the roots. A lower Ni return to soil is observable for litter treated plants because no strong recycling is needed since Ni available pool is already higher in surface soil.

Balance and fluxes of major nutrients

Ultramafic soils have relatively low concentrations of P, S, K and Ca compared to non-ultramafic soils in Sabah (van der Ent et al. 2016). Local hyperaccumulator plants (e.g. *P. rufuschaneyi* and *R. cf. bengalensis*) growing on ultramafic soils accumulate substantial concentrations of these essential nutrients in foliar tissues (van der Ent and Mulligan 2015). These elements are reabsorbed by the leaves before senescence, especially P. Potassium, however, leaches readily and correlates positively with leaf degradation (R^2 0.92; p 0.03), compared to Ca. Under high litter stock, plants tend to return less elements to the soil, except for Ca and P. Decrease in P stock due to harvesting can lead to a limitation of this nutrient in the soil for plant development. This element is often limited in tropical forest (Vitousek 1984). A low P fertilisation has to be done to avoid a decrease of biomass production in an agromining operation. The Ca budget is negative, with the soil concentrations decreasing rapidly under cultivation. It has been established that Ca amendment has a positive effect on the Ni yield of *P. rufuschaneyi* (Nkrumah et al. 2019b); and hence, it is likely that depletion decreases Ni yield in the long term. The increase of concentrations of K and Ca in aerial biomass are not desirable for

agromining applications because ashes need to be washed for the removal of this element. The K budget is negative if only litterfall input is taken into account. Strong K addition decreased Ni yield in pots (Nkrumah et al. 2019a, b). If the depletion of K was to continue at the same rate, moderate K fertilisation would be needed every three years. Litter input modifies translocation factors of Mg and Mn; when litter is present, these factors are reduced due to an increase of their concentrations in roots. Less Mn return to the soil by stemflow and less Mg and Mn return by litter input. Litter implied a lower translocation of Mn and Mg to aerial biomass and a lower return of this element to the soil. A tendency to have less Mg in the soil is visible. Soils under both litter treatments are more concentrated in K. Litter treatment increases exchangeable Na, P, S and K and the corresponding total concentrations. This effect was not shown on total soil concentration amended with *O. chalcidica* litter (Echevarria et al. 2021).

Implications for Ni agromining

The number of harvests needed to significantly deplete the initial labile Ni pools is still unknown and unpredictable in Pahu conditions after less than two years of experiments. Even under sustained agromining, litterfall is significant and represents 29% of Ni stock in biomass stand and 39% of harvested Ni outflux. Agromining operations harvest Ni-rich shoot biomass, which will limit Ni recycling through leaf litter. After almost two years of cropping, no decrease of Ni available pool has been recorded. Nickel litter fluxes combined with weathering could be sufficient to replenish Ni available pools under sustainable agromining employing *P. rufuschaneyi*. Biomass harvesting depletes essential nutrients, including P, S, K and Ca, as well as trace nutrients (*e.g.* Zn). Therefore, moderate fertilisation, especially P, would be required to avoid complete nutrients depletion in a sustainable Ni agromining.

This study highlights the biogeochemical cycling of Ni and physiologically relevant elements (P, S, K, Ca) in a tropical agromining metal farm. The Ni cycle of tropical hyperaccumulator plants is mainly driven by internal cycles. Nickel is returned to soil by litterfall even under sustained harvesting. After two years, no decrease of Ni pool was noticed. Tropical Ni agromining could be economically viable for several years, the exact timeframe is yet to be determined. Weathering and root uptake studies could predict the effect of Ni input and output flux on available Ni pool and thereby estimate the potential time scale of tropical Ni agromining. Furthermore, Ni stable isotope fractionation investigations could further clarify the processes involved in the Ni biogeochemical cycling in a wet tropical hyperaccumulator plants environment.

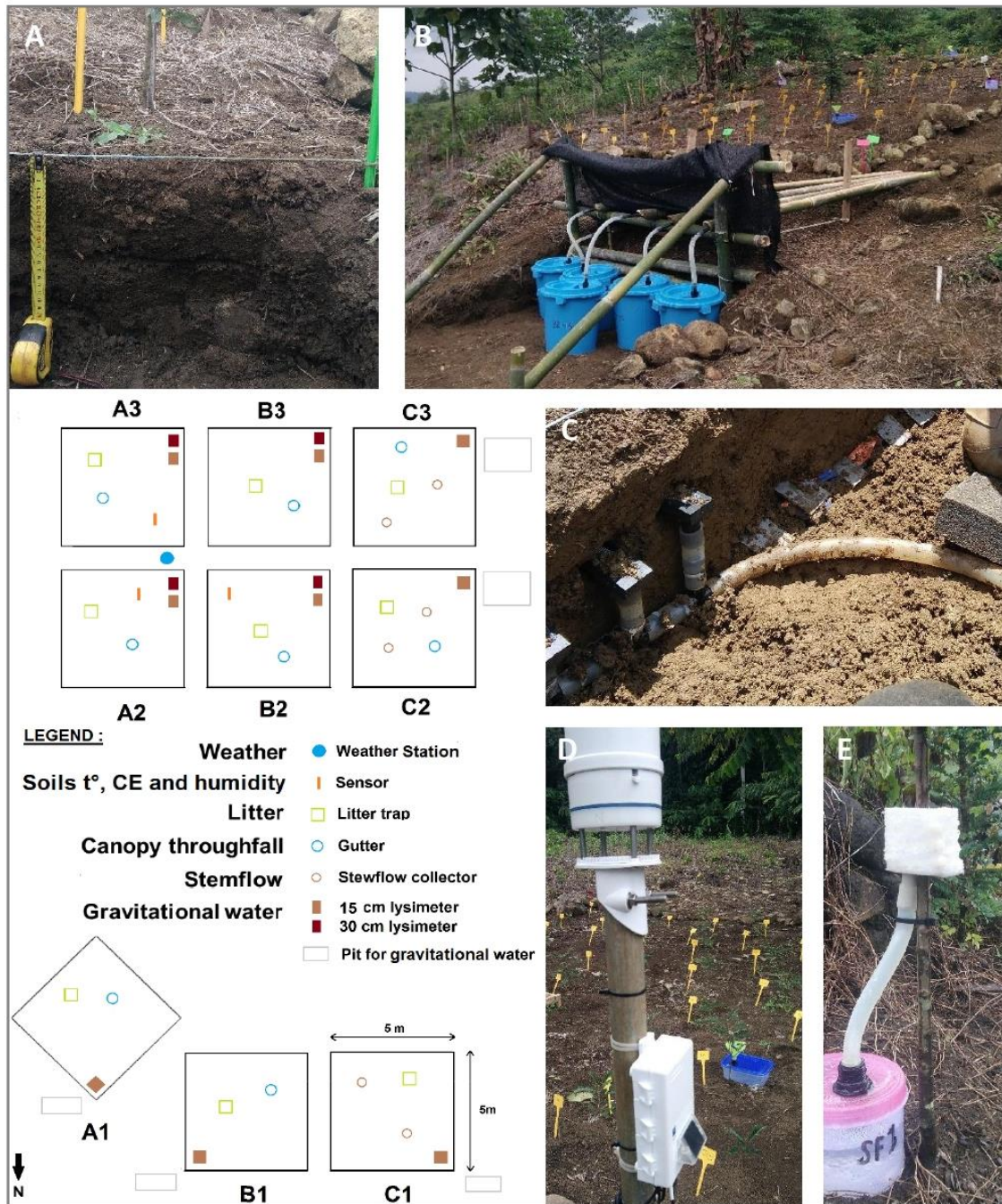
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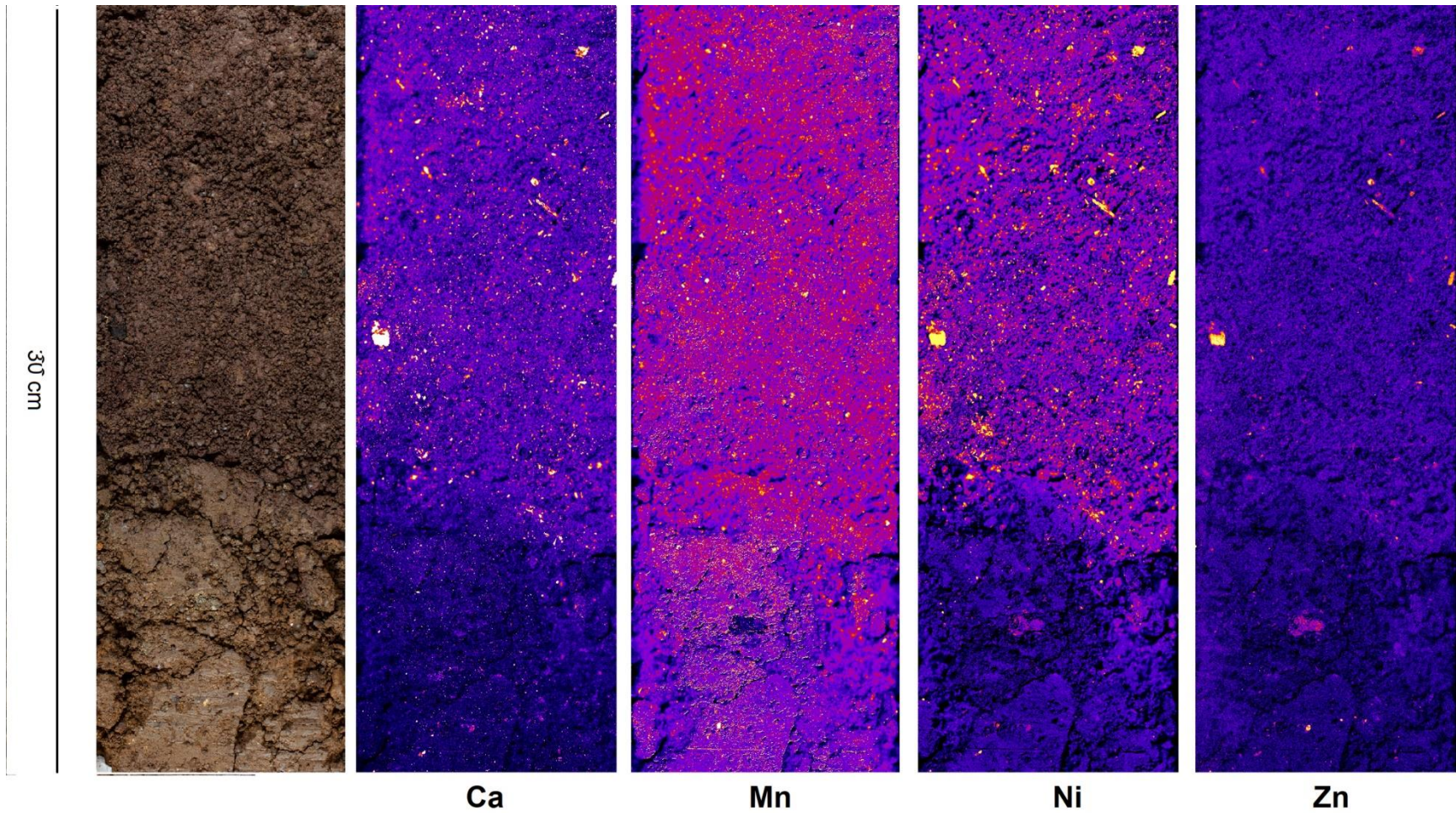
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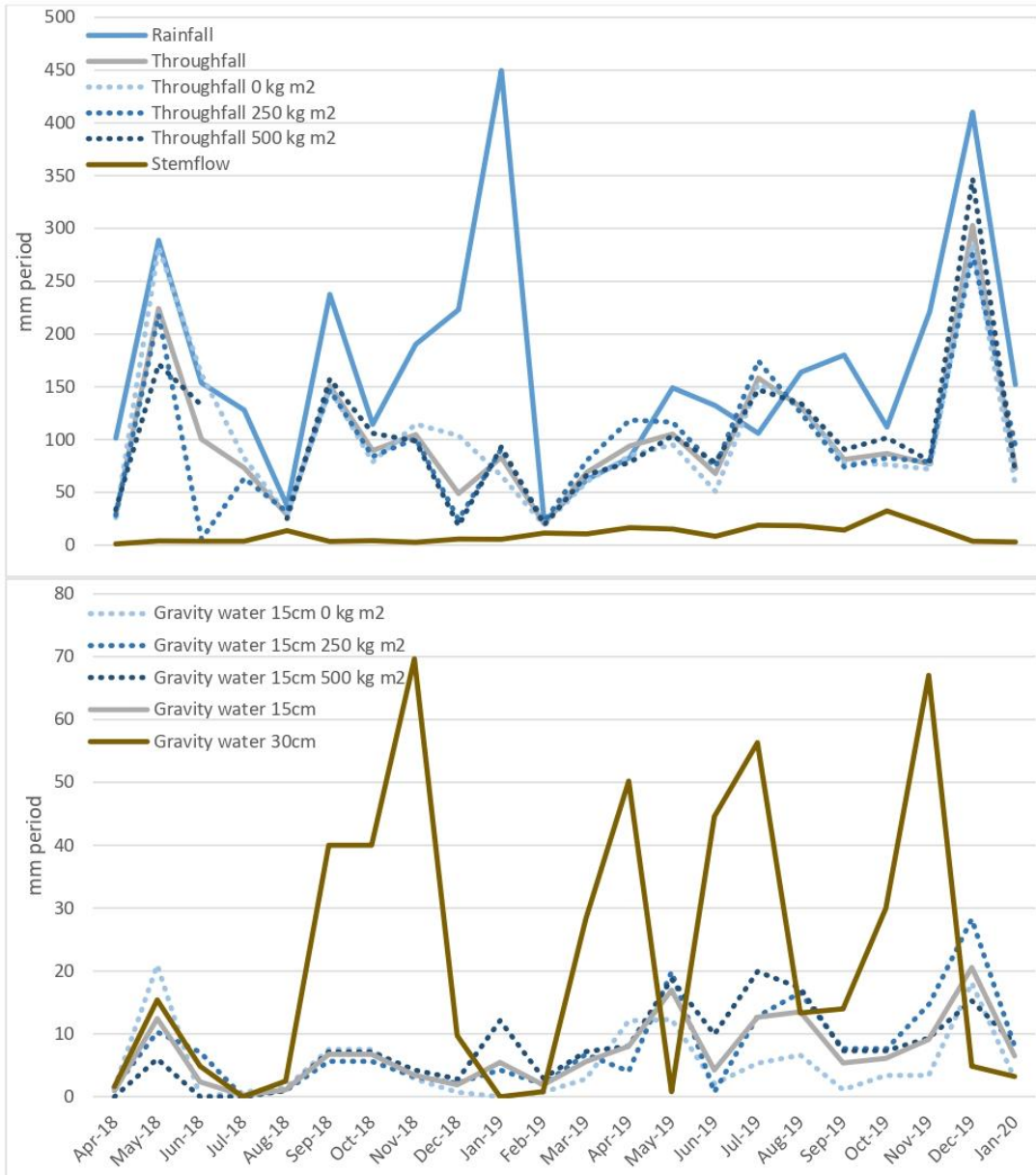
Supplementary Figure 5. Geochemical station of Pahu metal farm installed in March 2018. A: Topsoil profil; B and C: Lysimeters; D: Weather station; E: Stemflow collector.



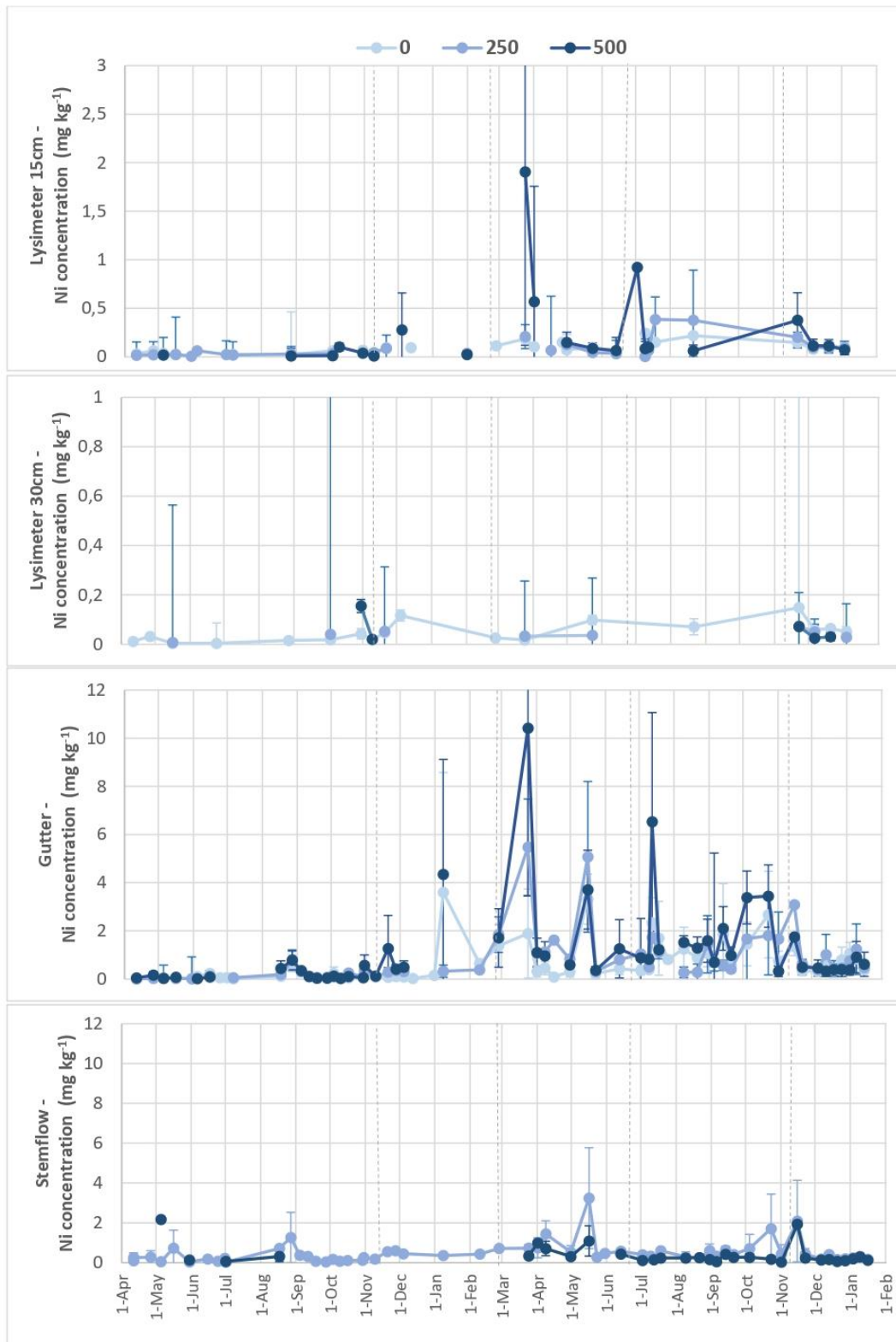
Supplementary Figure 6: microXRF scan of a soil core from a non-treated plot (B3).



Supplementary Figure 7. Water amount of the different instruments of the biogeochemical station from April 2018 to January 2020 in function of the litter treatments (Rainfall, Throughfall, Stemflow, Gravity water)



Supplementary Figure 8. Ni concentration of gravity water (15 to 30cm), throughfall and stemflow in mg kg^{-1} from April 2018 to January 2020



Supplementary Table 2. Total, DTPA, CEC and oxides concentrations of soils in November 2018 from 0 to 15cm depth in mg kg⁻¹ before first litter treatment. OxC: crystalline oxides, OxA: amorphous oxides; OxMn: Manganese oxides.

		Treatment kg m ⁻²	Ca	K	Mg	Ni	P	S	Mn
			mg kg ⁻¹						
Total	0-5cm	0	3400	260	54 000	4300	260	240	6000
		1	3400	300	54 000	4300	310	270	5400
		2	4000	320	54 000	4300	280	260	5300
	0-10cm	0	2700	190	55 000	4200	240	240	6600
		1	3700	200	56 000	4100	270	280	6000
		2	3800	220	56 000	4300	260	270	5500
	10-15cm	0	3200	220	54 000	4500	260	260	6200
		1	3300	220	53 000	4300	280	270	5500
		2	3400	220	53 000	4400	250	250	5600
DTPA	0-5cm	0				170			80
		1				170			90
		2				170			80
	0-10cm	0				160			100
		1				140			90
		2				160			90
	10-15cm	0				140			110
		1				120			100
		2				160			90
CEC	0-5cm	0	1500	70	1700	60			
		1	1300	70	1700	60			
		2	1700	80	1900	60			
	5-10cm	0	1300	60	1500	60			
		1	1100	60	1800	50			
		2	1500	40	1600	60			
	10-15cm	0	1000	60	1500	50			
		1	1000	60	1800	50			
		2	1500	60	1800	60			
OxC	0-5cm	0				1000			
		1				800			
		2				820			
OxA		0				2000			
		1				1600			
		2				1900			
OxMn		0				650			
		1				490			
		2				520			

Supplementary Table 3. Total, DTPA and CEC concentrations of soils in January 2020 from 0 to 5cm depth in mg kg⁻¹ in function of litter treatment applied. 4400

		Treatment kg m ⁻²	Ca	K	Mg	Ni	P	S	Mn
			mg m ⁻²						
Total	maximum	0	1100	50	18 000	2700	100	90	3500
	minimum		5700	250	71 000	7200	320	280	6200
	average		2700	140	47 000	5400	230	190	4770
	maximum	1	1200	90	22 000	3900	120	110	2490
	minimum		5200	410	82 000	7500	420	330	5800
	average		3000	250	48 000	5900	290	220	
	maximum	2	390	210	56 000	5700	460	200	<LOD
	minimum		6400	470	<LOD	8600	<LOD	520	5700
	average		3400	390	43 000	6500	340	280	4200
DTPA	maximum	0				110			40
	minimum					550			100
	average					200 a			70 a
	maximum	1				130			10
	minimum					800			130
	average					370 b			60 b
	maximum	2				140			1
	minimum					980			70
	average					430 b			40 c
CEC	minimum	0	1100	40	690	40			
	maximum		2200	140	2700	250			
	average		1700	80 a	1600	100 a			
	minimum	1	570	80	640	80			
	maximum		1800	240	3200	410			
	average		1300	150 b	1800	200 b			
	minimum	2	630	100	980	40			
	maximum		5200	290	3000	500			
	average		2000	220 c	2000	240 b			

Supplementary Table 4. Total, DTPA and CEC concentrations of soils in January 2020 from 10 to 15cm depth in mg kg⁻¹ in function of litter treatment applied.

		Treatment kg m ⁻²	Ca	K	Mg	Ni	P	S	Mn
			mg kg ⁻¹						
Total	maximum	0	950	40	37 000	3800	<LOD	100	3300
	minimum		17 000	170	120 000	95600	180	300	6200
	average		3500	90	55 000	5900	80	180	4900
	maximum	1	1200	90	34 000	4100	80	100	1900
	minimum		7100	480	84 000	11 000	400	500	8500
	average		3300	170	54 000	6100	170	240	4600
	maximum	2	640	80	36 000	4600	70	160	3100
	minimum		5800	410	65 000	8800	290	380	5900
	average		3100	220	50 000	5900	180	230	4400
DTPA	maximum	0				80			20
	minimum					280			110
	average					120			70
	maximum	1				40			20
	minimum					260			180
	average					140			90
	maximum	2				100			30
	minimum					320			140
	average					170			70
CEC	maximum	0	570	20	790	42			
	minimum		2400	100	3300	120			
	average		1400	60	1900	70			
	maximum	1	370	30	510	20			
	minimum		1200	190	3600	120			
	average		1100	80	2100	80			
	maximum	2	330	40	950	30			
	minimum		3400	1700	3700	280			
	average		1700	120	2100	110			

Supplementary Table 5. Element stocks of *P. rufuschaneyi* organs in January 2020 in function of the litter treatment applied in mg m⁻².

	Treatment mg m ⁻²	Ca	K	Mg	Ni	P	S	Mn
		mg						
Young leaves	0	170	170	100	460	30	60	6
	1	230	250	110	620	40	90	11
	2	310	330	100	670	50	90	8
Young stem	0	70	100	10	70	20	20	1
	1	90	130	20	70	30	20	2
	2	120	150	20	100	30	20	1
Old leaves	0	670	520	260	1900	70	180	20
	1	530	280	230	1500	50	150	20
	2	930	650	270	2000	80	220	20
Old stem	0	660	700	80	560	160	100	6
	1	720	870	110	790	250	170	7
	2	920	920	80	790	240	150	5
Aerial Total	0	1600	1500	450	3000	270	360	40
	1	1600	1500	460	3000	370	430	40
	2	2300	2000	470	3600	390	500	40
Primary roots	0	340	290	140	890	60	90	7
	1	720	320	160	1200	80	150	8
	2	840	530	210	1300	70	150	11

Supplementary Table 6. Fluxes in $\text{g m}^{-2}\text{y}^{-1}$ of elements from January 2019 to January 2020 in the function of the litter treatments applied.

Quantity $\text{g m}^{-2}\text{y}^{-1}$	litter kg m^{-2}	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Zn
Stemflow	1	0.02	-	0.01	0.01	-	-	-	-	0.20	-
	2	-	-	0.01	0.01	-	-	-	-	0.15	-
Throughfall	-	1.4	0.04	2.10	1.0	0.10	0.40	1.1	0.04	40	0.04
	1	1.4	0.03	2.10	0.70	0.10	0.40	1.4	0.02	50	0.05
	2	1.8	0.04	2.70	1.0	0.10	0.30	2.2	0.06	52	0.05
Litterfall	-	0.6	0.12	0.10	0.20	0.03	-	3.0	0.03	0.08	0.02
	1	0.4	0.05	0.10	0.20	0.02	-	1.4	0.02	0.05	0.01
	2	0.6	0.05	0.10	0.10	0.02	-	1.6	0.02	-	0.06
Gravity water 15cm	-	0.4	0.01	0.10	0.60	-	0.03	0.01	-	4.9	-
	1	0.3	0.06	0.10	0.60	-	0.05	0.02	-	7.4	-
	2	0.9	0.03	0.10	1.1	-	0.07	0.02	-	8.5	-
Gravity water 30cm	0	0.3	0.11	0.10	1.2	-	0.06	0.01	-	4.3	-
	1	0.3	0.01	0.05	0.40	-	0.02	-	-	1.5	-
	2	0.6	-	0.10	0.50	-	0.04	-	-	5.30	-

Supplementary Table 7. Percentage remaining (%) and quantity remaining (mg kg⁻¹) of elements in litter bags collected from 1 to 15 months after their implantation.

			01/11/2018	01/12/2018	31/01/2019	22/04/2019	01/08/2019	01/02/2020
Pourcentage remaining (%)	Ni	leaflets	100	90	70	120	90	50
		petiole	100	110	90	100	80	60
		wood	100	120	100	170	100	
	Zn	leaflets	100	130	100	80	140	100
		petiole	100	170	130	140	170	140
		wood	100	260	110	100	120	
	P	leaflets	100	90	90	90	90	90
		petiole	100	40	40	40	40	20
		wood	100	30	40	60	30	
	S	leaflets	100	90	90	100	110	90
		petiole	100	130	110	120	100	80
		wood	100	150	70	100	80	
K	leaflets	100	20	10	0	0	0	
	petiole	100	10	10	0	0	0	
	wood	100	20	0	0	0		
Ca	leaflets	100	130	120	130	110	130	
	petiole	100	130	140	130	110	90	
	wood	100	190	130	160	130	-	
Quantity remaining (mg kg ⁻¹)	Ni	leaflets	22100	24800	20200	33200	33400	16800
		petiole	13900	14600	11500	14700	14500	13900
		wood	5800	6900	6000	11100	6500	11800
	Zn	leaflets	180	300	240	190	460	290
		petiole	150	230	180	210	340	330
		wood	70	180	80	70	90	270
	P	leaflets	900	1000	1000	1000	1300	1200
		petiole	2200	900	1000	1000	1100	900
		wood	1400	400	500	800	500	900
	S	leaflets	1500	1700	1600	1900	2700	2000
		petiole	1000	1300	1100	1300	1500	1400
		wood	700	1000	500	700	600	1200
	K	leaflets	6600	1300	800	<LOD	<LOD	<LOD
		petiole	12100	1000	1400	<LOD	<LOD	<LOD
		wood	6600	1100	<LOD	<LOD	<LOD	<LOD
	Ca	leaflets	4600	7400	6900	7400	9200	8900
		petiole	5300	6500	6900	7500	7900	7500
		wood	3800	7100	4800	6300	5400	7100

CHAPTER 4: Improving Tropical Ni Agromining with fertilizer

Improving tropical nickel agromining crop systems: the effects of chemical and organic fertilisation on nickel yield

Romane Tisserand^{1,2}, Antony van der Ent^{1,2}, Philip Nti Nkrumah²,
Sukaibin Sumail³, Guillaume Echevarria^{1,2*}

¹Université de Lorraine, INRAE, Laboratoire Sols et Environnement, 54000 Nancy, France.

²Centre for Mined Land Rehabilitation, Sustainable Minerals Institute, The University of Queensland, Australia.

³Sabah Parks, Kota Kinabalu, Sabah, Malaysia.

*Corresponding author: Guillaume Echevarria

ABSTRACT

Aims The agronomy of tropical ‘metal crops’ had not yet been tested until a recent pot trial was undertaken to test the growth and nickel (Ni) yield of two promising Ni ‘metal crops’ (*Phyllanthus rufuschaneyi* and *Rinorea cf. bengalensis*) from Sabah, Malaysia. The present study investigates the growth and Ni yield in *P. rufuschaneyi* in response to inorganic and organic fertilisation under field conditions.

Methods A two-year field experiment was undertaken on an ultramafic Cambisol in Sabah. Two experiments were set up with different types of fertilisers, either organic or inorganic. Ten 9-m² plots were amended with either NPK (370 kg ha⁻¹) or N (120 kg ha⁻¹) fertilisation and were monitored during the first year. Another experiment with 25-m² plots was set up to test three treatments of organic amendment (0, 1 or 2 kg m⁻² of shredded *P. rufuschaneyi* biomass).

Results The results show that despite considerable variability, Ni concentration was generally lower in plant shoots amended with N only (*i.e.* urea). However, treatments showed no statistical effect on plant biomass and Ni yield in the short-term, even though organic treatments had a strong positive effect on the availability of Ni.

Conclusion The short-term effects of inorganic and organic treatments on Ni yield of the tropical ‘metal crop’ *Phyllanthus rufuschaneyi* were not apparent (75 kg Ni ha⁻¹ yr⁻¹). The field trials are ongoing to assess long-term effects. The preliminary results suggest that with appropriate agronomic systems and plant breeding, the Ni yield could be substantially increased.

Keywords: Agromining, hyperaccumulator, ultramafic, nickel, fertilisation.

A. INTRODUCTION

Southeast Asia has some of the largest ultramafic outcrops globally, covering an area of 23 000 km² (van der Ent et al. 2013a; Galey et al. 2017). The state of Sabah (Malaysia) has extensive ultramafic outcrops, covering 4.6% (3500 km²) of the land area of northern part of Borneo (Proctor et al. 1988; Repin 1998). These soils are characterised by low concentrations of major nutrients (*e.g.* P and K) and high concentrations of potentially toxic metals (*e.g.* Ni, Co, Fe and Mn) (Echevarria 2018). These inherent soil properties present edaphic challenges to most plants. However, ultramafic soils support plant species which have evolved ecophysiological mechanisms to either exclude or uptake potentially toxic metals into their living shoots (Baker 1981). Hyperaccumulators are plants that have evolved naturally to accumulate high concentrations of metals in their shoot biomass (Reeves et al. 2003; van der Ent et al. 2013b). Currently, more than 700 metal hyperaccumulator plant species are recorded in the world, of which >500 hyperaccumulate Ni with foliar Ni concentrations >0.1% (Reeves et al. 2018). In Southeast Asia, there are ~20 and ~50 known Ni hyperaccumulator plant species in Halmahera (Indonesia) and Sabah, respectively (Lopez et al. 2019b; van der Ent et al. 2019). Hyperaccumulators hold much promise for fundamental research (*e.g.* metal regulation in plants) (Nkrumah et al. 2018a, b; van der Ent et al. 2017) and practical green technology (*e.g.* mine site rehabilitation) (Erskine et al. 2018).

Agromining is a chain of processes that consists of growing, harvesting, drying and incinerating selected hyperaccumulator plants to recover a high-grade bio-ore that can be processed for metallurgical purposes (Barbaroux et al. 2011; Chaney et al. 2007; Nkrumah et al. 2018c). Nickel agromining is simultaneously possible on low-productive ultramafic soils and on degraded or mined land (van der Ent et al. 2013b, 2015a). The desirable traits of candidate species for economic Ni agromining include high biomass production and ability to accumulate more than 1 wt% Ni in their shoot ('metal crops') (Chaney et al. 2007; Nkrumah et al. 2016a). Agronomic systems based on insights from laboratory and field studies are proposed to maximise Ni yield for viable agromining operations (Bani et al. 2007; Broadhurst and Chaney 2016; Kidd et al. 2018; Li et al. 2003a; Nkrumah et al. 2018d). Inorganic fertiliser application, weed control, optimum plant density, optimum soil pH and adequate soil drainage increase Ni yield of *Odontarrhena chalcidica* (Li et al. 2003a; Bani et al. 2015). Organic matter amendments generally decrease extractable Ni concentrations, but increase biomass production, and may increase or decrease Ni yield depending on shoot Ni concentrations (Alvarez-Lopez

et al. 2016; Bani and Echevarria 2019; Chaney et al. 2016). In a recent field trial, Bani and Echevarria (2019) demonstrated that in a high extractable Ni ultramafic soil, combining inorganic and organic fertilisation improves Ni yield of *O. chalcidica*, reaching 145 kg Ni ha⁻¹, compared to using only inorganic fertilisation, attaining 105 kg Ni ha⁻¹ (Bani et al. 2015). These field trials are critical to develop a sustainable commercial agromining operations. Although, unrealised opportunities exist for economic Ni agromining in the tropics, notably in Sabah (Malaysia), Halmahera (Indonesia) and Palawan (Philippines), the agronomic systems have not been tested in the field (van der Ent et al. 2013b; Nkrumah et al. 2016b; 2018c, d).

The first tropical ‘metal farm’ has been established in Sabah in an ultramafic Cambisol derived from a strongly serpentinised bedrock to test the viability of Ni agromining in the tropical context (Nkrumah et al. 2019a). Tropical ultramafic Cambisols are characterised by circum-neutral pH and high Ni DTPA (>150 mg kg⁻¹ Ni) (van der Ent et al. 2016; Echevarria 2018). The two most promising tropical ‘metal crops’ in Sabah are *Phyllanthus rufuschaneyi* (Phyllanthaceae) and *Rinorea cf. bengalensis* (Violaceae) (Nkrumah et al. 2016a, 2018d; van der Ent et al. 2015a). These species have high biomass production and shoot Ni concentrations (>1 wt% Ni) (Bouman et al. 2018; van der Ent and Mulligan 2015). Recent pot trials have shown that the Ni yield of these ‘metal crops’ can be improved under appropriate agronomic system (Nkrumah et al. 2019b, c, d). Inorganic fertilisation (N, P and K) and organic matter amendments have strong positive effect on the growth of *R. cf. bengalensis*. For *P. rufuschaneyi*, only N and P have strong positive growth effects, whereas both K and organic matter (OM) additions have adverse growth effects, especially OM. Calcium and S additions have no significant effects on the growth of both *P. rufuschaneyi* and *R. cf. bengalensis* but improve Ni uptake in these species. Organic matter amendments significantly reduce shoot Ni concentrations in both species and do not appear to be useful in economic tropical agromining when employing these species on Cambisol, whereas Ca, S and P additions are essential to improve tropical agromining (Nkrumah et al. 2019b, c). High extractable Ni concentrations and circum-neutral soil pH increase Ni yield of *P. rufuschaneyi* and *R. cf. bengalensis* (Nkrumah et al. 2019d). These observations from the pot trials provide critical insights into developing tropical Ni agromining. However, prior to commercial large-scale Ni agromining operations, field trials are needed to develop these agronomic systems under ‘real-life’ field conditions.

This study investigates the Ni uptake and growth responses of *P. rufuschaneyi* to increased additions of nutrients (N, P and K) and litter under realistic field conditions for a two-year period.

B. MATERIALS AND METHODS

a. Plant species used

This study selected *P. rufuschaneyi* because of its highly desirable traits for agromining purposes. *Phyllanthus rufuschaneyi* accumulates exceptional concentrations of Ni in its shoots, with up to 2.5 wt% in old leaves and 1.2 wt% in twigs (van der Ent et al. 2015b; van der Ent and Mulligan 2015; Nkrumah et al. 2019a). The Ni concentrations in the phloem sap are high, with mean concentrations of 8800 $\mu\text{g g}^{-1}$ (van der Ent and Mulligan 2015). Considering the global high shoot Ni concentrations, rapid re-growth after coppicing, tolerance for exposed conditions and pest resistance, *P. rufuschaneyi* is a strong candidate for Ni agromining in the tropics (Bouman et al. 2018; Nkrumah et al. 2019a). This recently described species is known to be restricted in the wild to only two localities in Sabah close to Kinabalu Park (*i.e.* Bukit Hampuan and Lompoyou Hill), making this plant extremely rare (Bouman et al. 2018). The soil type in its native habitat is a young (eroded) hypermagnesian Cambisol on strongly serpentinised bedrock (van der Ent et al. 2018). *Phyllanthus rufuschaneyi* flowers and fruits all year round (Bouman et al. 2018) and is likely pollinated by *Epicephala* moths (Kato and Kawakita 2017). Interestingly, the reproductive organs (flowers, fruit capsule and seeds) are highly Ni-enriched, with seeds and flowers reaching 1.76 and 0.56 Wt% Ni, respectively (Bouman et al. 2018).

Experimental set up

This study was undertaken in a large ultramafic field in Pahu, Sabah, Malaysia (335m asl; 6°7'49"N;116°46'22"E). This Eutric Chromic Cambisol, which is similar to many saprolite mine wastes, has been described previously (Nkrumah et al. 2019a). Wild saplings of *P. rufuschaneyi* were collected from their native ultramafic habitats and established in polyethylene bags containing ultramafic soils from the Pahu field in a 70% shade-cloth housing. The saplings were then transplanted into the field plots in February 2018 for the organic matter amendment experiments, and in November 2018 for the chemical amendment experiments. Two plot sizes were established (Figure S8, S9): i) 25 m² plots (9X) were planted with *P. rufuschaneyi* at a density of two plants per square meter, and ii) 9 m² (10X) plots planted with *P. rufuschaneyi* at a density of four plants per square meter.

Treatments application

After transplanting, plants were allowed to establish on the plots for a period of eight months before organic matter (OM) application. We wanted to avoid the effect of the treatment being overshadowed by the effect of the adaptation of the transplanted saplings to its new environment. Each OM treatment had three replicates. All OM treatments received an initial homogeneous NPK fertilisation (370 kg ha^{-1}), which was applied once prior to the application of the OM treatments (Table 10, Table 11, Table 12). The OM was composed of *P. rufuschaneyi* shoot biomass crushed with a shredder (1–3 cm particle size); the bulk elemental concentrations are given in Table 12. The OM treatment was applied at three different rates: 0, 250 or 500 g m^{-2} at each application. Each OM treatment was applied four times (every four months, *i.e.* early November 2018, Mid-March, End-June and early November 2019) over the duration of the experiment, thus translating to 0, 1 and 2 kg m^{-2} in total (Figure 22).

All the inorganic treatments were applied in November 2018. Soil inorganic treatments were applied only once and included an untreated control (no amendment). Treatments with inorganic fertilisation included NPK 15-15-15 slow release granules (370 kg ha^{-1}) or N granules alone (120 kg ha^{-1}), applied manually on the soil surface.

Biomass measurement and analyses

The growth parameters of each plant were measured every month (plant height, lignification height, height of the first leaves, diameter of main stems, number of branches, presence of fruits and seedlings). For the OM treatments, 12 plants per treatment were collected 14 months after initial OM treatment application, along with their rhizosphere soil. Plants were then separated into different fractions: roots, lower stems, upper stems, old leaves and young leaves. Roots were washed with ultra-pure water. Roots and shoots were dried at $40 \text{ }^{\circ}\text{C}$ in a drying oven for 48 h prior to grinding. Concentrations of total elements in plants were carried out on 50-mg powdered plant samples which were digested with 1 mL HNO_3 and 2 mL H_2O_2 . After 16 h, samples were placed in a DigiPREP® system for 120 min at $95 \text{ }^{\circ}\text{C}$ and then filtered at $0.45 \text{ }\mu\text{m}$ before analysis with ICP-OES (Thermo Fisher iCAP 6300 Duo).

Table 10 Total and extractable trace elements concentrations in the soil of the respective treatments of *Phyllanthus rufuschaneyi* before application (n=12) The elemental concentrations are given in means \pm standard error in mg kg⁻¹ Extracts were analysed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES)

Treatments	Pseudo Total				DTPA Ni
	Cr	Fe	Ni	Zn	
	mg kg ⁻¹				
Organic control	6100 (\pm 1600)	170 000 (\pm 23 000)	4600 (\pm 140)	150 (\pm 14)	160 (\pm 17)
1 kg litter	5100 (\pm 1200)	170 000 (\pm 13 000)	4000 (\pm 470)	153 (\pm 13)	150 (\pm 40)
2 kg litter	5000 (\pm 900)	170 000 (\pm 18 000)	4300 (\pm 160)	157 (\pm 17)	160 (\pm 32)
Inorganic control	5500 (\pm 1400)	155 000 (\pm 11 000)	3400 (\pm 460)	140 (\pm 13)	90 (\pm 25)
NPK	5900 (\pm 890)	167 000 (\pm 18 000)	3600 (\pm 480)	160 (\pm 10)	120 (\pm 30)
Urea	4700 (\pm 691)	147 000 (\pm 11 000)	3400 (\pm 460)	140 (\pm 8)	110 (\pm 45)

Table 11 Total and bioavailable nutrients concentrations in the soil of the respective treatments of *Phyllanthus rufuschaneyi* before application (n=12) The elemental concentrations are given in means \pm standard error in mg kg⁻¹ Extracts were analysed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES)

Treatments	pH	Pseudo Total					P Olsen	CEC		
		C	Ca	K	Mg	N		Ca	K	
	mg kg ⁻¹								cmol ⁺ kg ⁻¹	
Organic control	6.7 (\pm 0.1)	28 (\pm 10)	3400 (\pm 1700)	260 (\pm 82)	54 000 (\pm 11 000)	3.4 (\pm 1.2)	6.9 (\pm 1.3)	1500 (\pm 600)	67 (\pm 24)	35 (\pm 8)
1 kg litter	6.5 (\pm 0.1)	32 (\pm 13)	3400 (\pm 1300)	300 (\pm 113)	54 000 (\pm 10 000)	3.4 (\pm 0.9)	8.5 (\pm 2.3)	1300 (\pm 400)	70 (\pm 33)	30 (\pm 4)
2 kg litter	6.5 (\pm 0.1)	35 (\pm 20)	4000 (\pm 1500)	320 (\pm 91)	54 000 (\pm 6000)	3.1 (\pm 0.7)	7.1 (\pm 0.5)	1700 (\pm 900)	79 (\pm 26)	37 (\pm 4)
Inorganic control	6.4 (\pm 0.1)		4600 (\pm 2000)	300 (\pm 130)	50 000 (\pm 4000)		6.5 (\pm 2.1)	1800 (\pm 880)	53 (\pm 26)	35 (\pm 8)
NPK	6.4 (\pm 0.1)		3800 (\pm 1100)	310 (\pm 130)	47 000 (\pm 2200)		7.0 (\pm 0.2)	1500 (\pm 390)	60 (\pm 27)	30 (\pm 4)
Urea	6.4 (\pm 0.1)		5153 (\pm 2000)	270 (\pm 92)	52 000 (\pm 5500)		8.8 (\pm 0.8)	1800 (\pm 760)	47 (\pm 17)	36 (\pm 4)

Soil collections and analyses

Surface soil samples were taken before treatment application every four months for the organic treatments, whereas that of the inorganic treatment were collected one year after treatment application. For the OM treatments, the 12 rhizosphere soils per treatment which were collected 14 months after initial OM treatment application, together with the plants were characterised. Physico-chemical analyses were performed on air-dried and sieved soil samples (2-mm sieve; NF ISO 11464). Soil pH and electrical conductivity were measured in a 1:5 soil: water mixture (NF ISO 10390). Total and organic C and N were quantified using a CHNS analyser according to the norms NF ISO 10694 and 13878. The cation exchange capacity (CEC) was determined spectrophotometrically (ISO/DIS 23470) by mixing 2.5 g of soil in 25 mL of a 0.05 M hexaamminecobalt(III) trichloride solution. After mechanical shaking (1 h at 20 °C), the hexaamminecobalt(III) trichloride concentration remaining in solution was measured using a spectrophotometer (absorption at 475 nm). Exchangeable trace elements were extracted with DTPA (diethylene triamine pentaacetic acid) with soil/solution ratio 1/2 (w/v) at pH 7.3 (NF ISO 31- 121). Extraction with aqua regia was performed on 0.5-g soil samples which were digested with 2 mL HNO₃ and 6 mL HCl. After 16 h, samples were placed in a DigiPREP® system (SCP Science, Baie-d'Urfé, QC, Canada) for 120 min at 105 °C and the resulting solutions were diluted to 50 mL with ultrapure water then filtered at 0.45 µm before analysis with ICP-OES.

Table 12 Composition of the treatment levels used in the growth trial in kg ha⁻¹.

Treatment	Treatment details		Composition (kg ha ⁻¹)					
	Organic	Inorganic	Ca	K	N	Ni	P	S
Organic control	–	370 kg ha ⁻¹ NPK	–	54	54	–	54	7
1 kg litter	1 kg m ⁻²		70	84	>54	100	59	9
2 kg litter	2 kg m ⁻²		140	110	>54	200	64	11
Inorganic control	–	–	–	–	–	–	–	–
NPK		370 kg ha ⁻¹ NPK	–	54	54	–	54	7
Urea		120 kg ha ⁻¹ Urea	–	–	55	–	–	–

Statistical analyses

Statistical analyses were performed using Xlstat (version 2019.3.2). Data did not fit parametric assumptions. The significant difference between treatments was tested using Kruskal-Wallis test with a confidence level of 95%. Significant differences were explored by Dunn's multiple pairwise comparison post-hoc test and results were adjusted using the Bonferroni correction.



Figure 22 Status of the field trial and litter treatments after the second application, 1 year after planting.

C. RESULTS

a. Effects of organic and inorganic treatments on nickel concentrations in soil

Almost eight months after the first cropping and before treatments were applied, no effect of the plant cover on both total Ni content and available Ni in soils was detectable at the surface soil (Figure 23). One year after the OM treatment applications started, differences between treatments were significant for DTPA-Ni ($p < 0.05$). There was a two-fold increase in Ni-DTPA in the topsoil of the 2 kg OM m⁻² treatment (420 mg kg⁻¹ Ni), compared to the control (no OM addition) after 11 months of initial OM treatment application (Figure 23). For the 1 kg OM m⁻² treatment, Ni availability increased slightly compared to the control after 11 months of initial OM treatment application. However, DTPA-Ni in the 1 kg OM m⁻² treatment was statistically similar to that of the 2 kg OM m⁻² treatment after 14 months of initial OM treatment application, with a two-fold increment compared to the control plots. For the rhizosphere soils, Ni availability was significantly higher in the 2 kg OM m⁻² treatment (145 mg kg⁻¹) relative to the control (105 mg kg⁻¹). However, DTPA-Ni concentrations of the rhizosphere soils were

statistically lower than the DTPA-Ni of the surface soils for each of the OM treatment ($p < 0.01$). The DTPA-Ni of the inorganic treatment was lower than that of the organic treatments. No effect of the inorganic treatment on DTPA-Ni concentrations could be detected among treatments during the entire time period ($p > 0.64$).

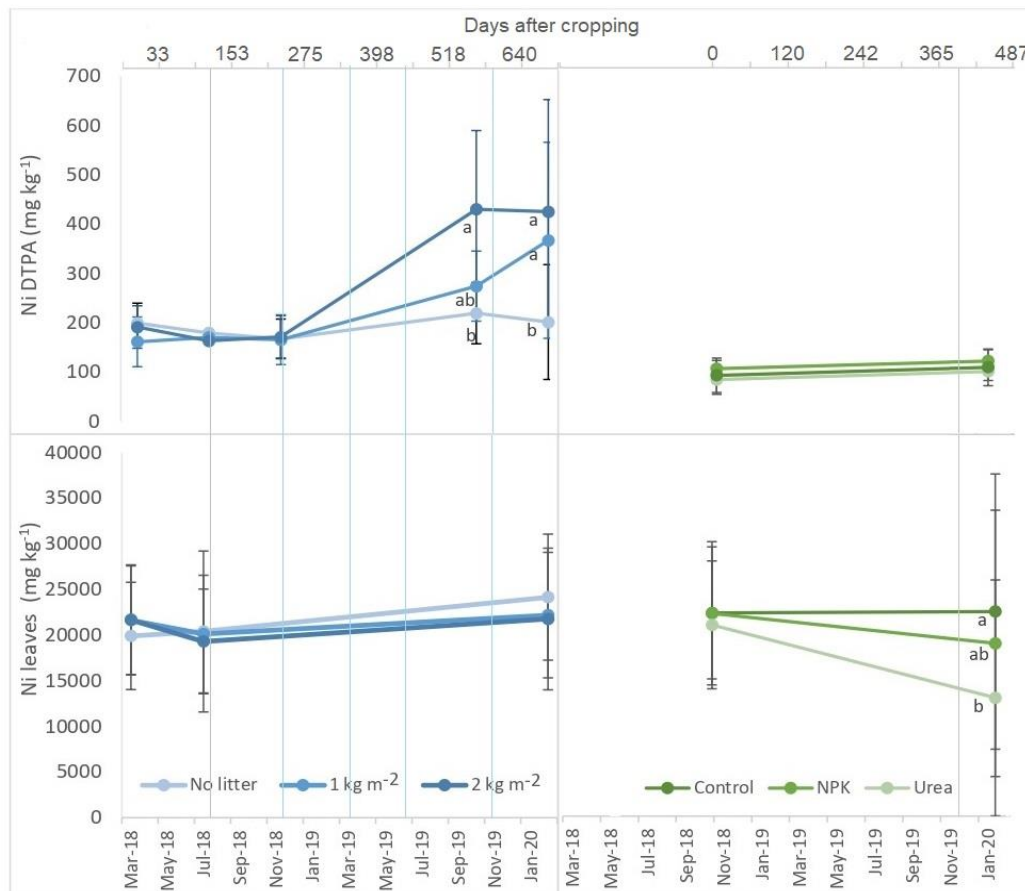


Figure 23 Nickel DTPA concentrations ($n=12$) and Ni concentrations in the leaves of *Phyllanthus rufuschaneyi* ($n=75$) over time. The elemental concentrations of organic and inorganics treatments are given in mg kg^{-1} . Grey lines indicate harvesting without treatment, and blue lines indicate harvesting with treatment

Nickel in *P. rufuschaneyi* in response to organic and inorganic treatments

Organic matter addition had no significant effect on relative Ni concentrations in leaves ($p = 0.78$) (Figure 23). Leaves of plants in the 2 kg OM m^{-2} treatment had lower Ni concentrations before and after treatment application relative to the plants in the 1 kg OM m^{-2} treatment and the control. For the chemical treatments, a decrease in the shoot Ni concentrations of plants amended with urea was the only significant effect (1.3 wt% Ni). Shoots from this treatment contained 32% lower Ni in their leaves compared to that of the non-fertilised control (1.9 wt% Ni) ($p=0.01$). Nickel concentrations in leaves were higher in the OM treated plots (2.0 wt% Ni) relative to inorganic fertiliser treated plots. The concentrations of Ni in the plants in the 2 kg OM m^{-2} treatment was 42% higher than that of the urea treatment after 14 months of the respective treatment applications ($p < 0.01$) (Figure 23).

Growth responses of *P. rufuschaneyi* in response to organic and inorganic treatments

Organic matter amendments had positive effects on the height of *P. rufuschaneyi*, and the increase corresponded to the rainfall intensity from 2.1 to 8.0 cm per month (Figure 24). The increase in height tended to be more pronounced in the 2 kg OM m⁻² treatment, notably in September 2019, but the difference was not significant ($p = 0.32$) (Figure 25). There were no statistical differences in the diameter growth (girth), the number of branches and the flowering between OM treatments. However, the number of branches and diameter seemed to be higher for non-treated control compared to the 2 kg OM m⁻² treatment ($p: 0.26$ and 0.16 , respectively).

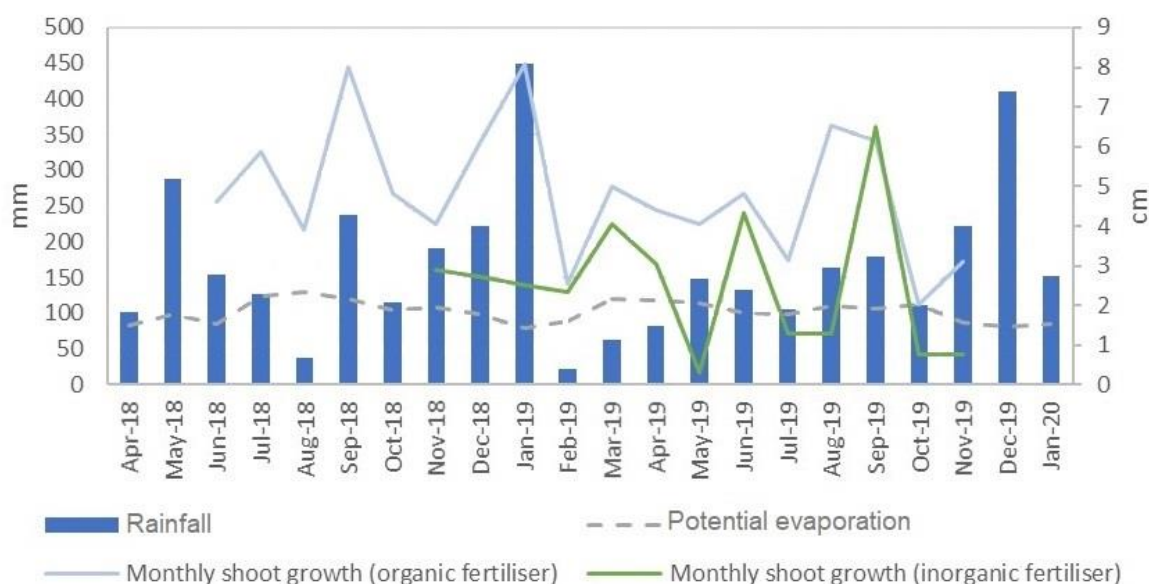


Figure 24 Monthly rainfall, potential evaporation and shoot growth of the plants amended with organic fertilisation ($n=225$) and with inorganic fertilisation ($n=33$).

There was positive effect on the growth in height of *P. rufuschaneyi* in response to inorganic treatment, increasing 0.2–6.2 cm per month (Figure 24). The effect of chemical fertilisation on the stem diameter and number of branches of *P. rufuschaneyi* was not visible directly after the application (Figure 25) but became more evident after nine months. There was a significant decrease in plant growth in the urea treatment compared with non-amended treatment after nine months of treatment application ($0.01 < p < 0.035$). The girths of plants treated with urea were statistically lower than those of the non-treated plants after 10 months of treatment application ($0.01 < p < 0.03$). The number of branches of the plants fertilised with urea was two-fold lower than the plants with no fertilisation after 11 months of treatment application ($0.001 < p < 0.02$). The NPK input had no statistical effect on the growth parameters of *P. rufuschaneyi* ($p = 0.27$) or on the percentage of flowering.

The period of lower and higher growth seemed to vary consistently and simultaneously among treatments and control (Figure 24). Growth was lower during dry months (especially February) and higher after significant rainfall (e.g. September). The relative growth in height of plants amended with inorganic fertiliser only was always lower relative to all other treatments, including the control of OM treatments ($p < 0.03$). However, the stem diameter (girth) seemed to be similar after one-year of cultivation between both the control in the OM treatment and NPK treatment ($p = 0.42$) (Figure 25).

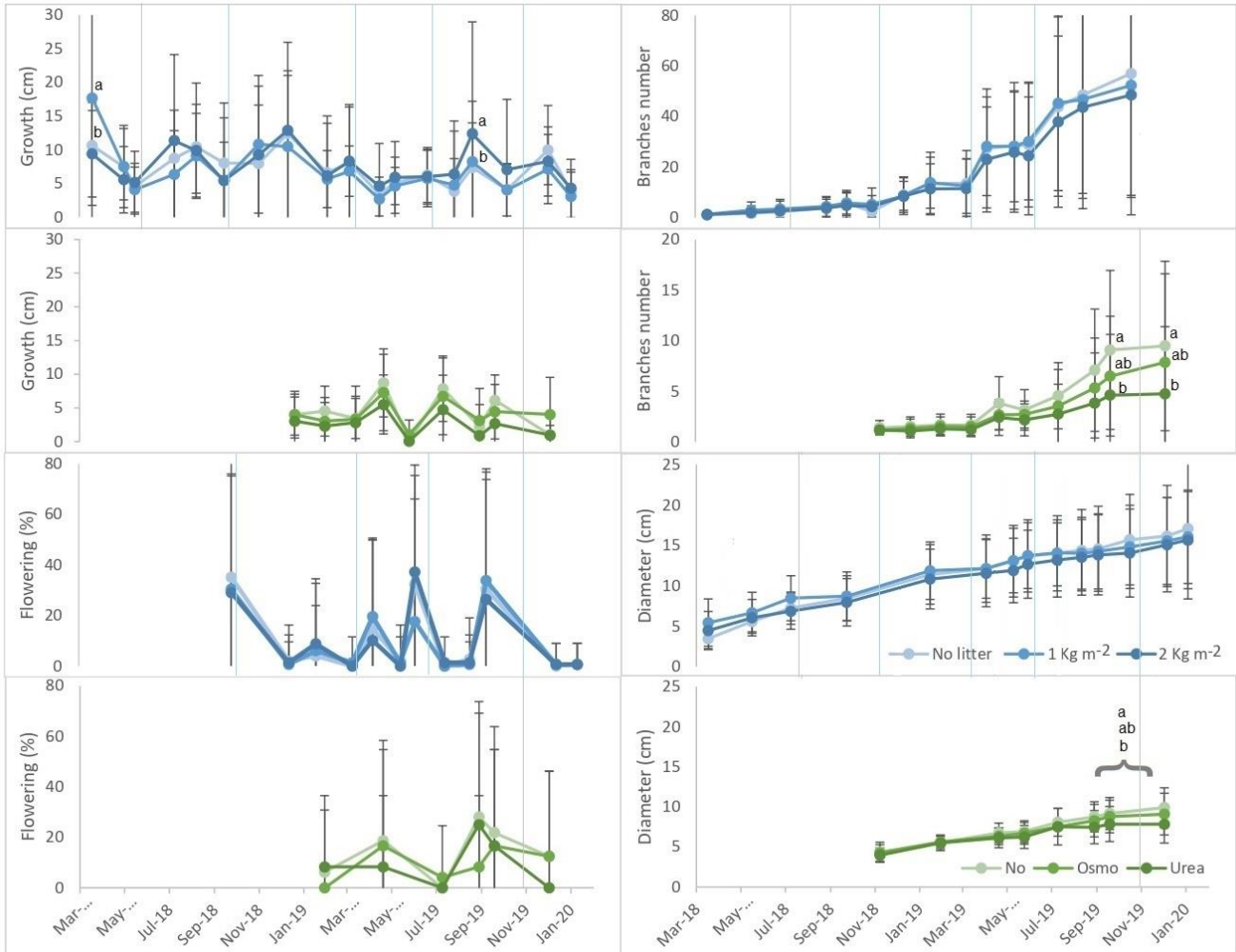


Figure 25 Changes in agronomic parameters over time of the plants amended with organic fertilisation ($n=225$) and with inorganic fertilisation ($n=33$) (shoot growth in cm, flowering in %, number of branches and diameter growth). Grey lines indicate harvesting without treatment, and blue lines indicate harvesting with treatment.

Biomass and nickel yield of *P. rufuschaneyi* under organic and inorganic treatments

For the OM treatment, four plants per plot (12 per treatment) were collected after 14 months of initial OM treatment application in order to establish a nutrient-based mass balance for each treatment (Figure 26, Figure 27, Table S9). The total biomass ranged dramatically from 35–600 g per plant, with different plant organs reaching 200 g for roots, 220 g for stems and 250 g for leaves. No statistical variation of the biomass weight of plants was observed among the OM

treatments. However, the total plant biomass in the 2 kg OM m⁻² treatment tended to be higher than that of the non-treated plots ($p = 0.16$). Organic amendments had a negligible effect on plant growth and shoot Ni concentrations compared to the control.

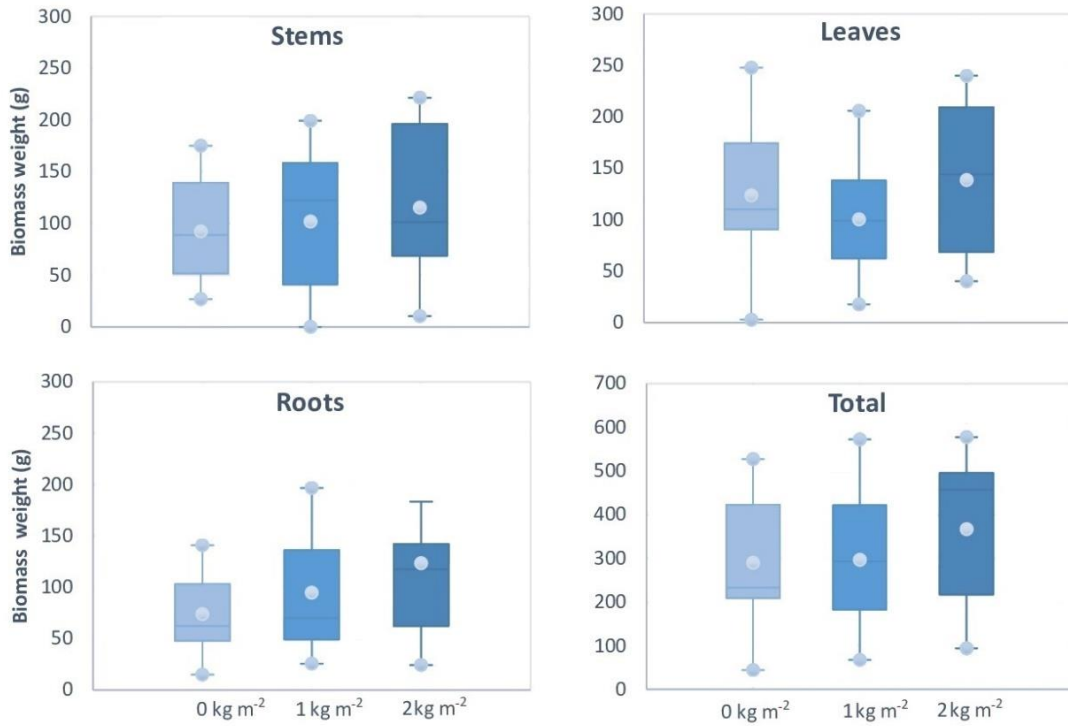


Figure 26 Biomass of the different organs of *P. rufuschaneyi* in the organic treatments in g per plant ($n=12$)

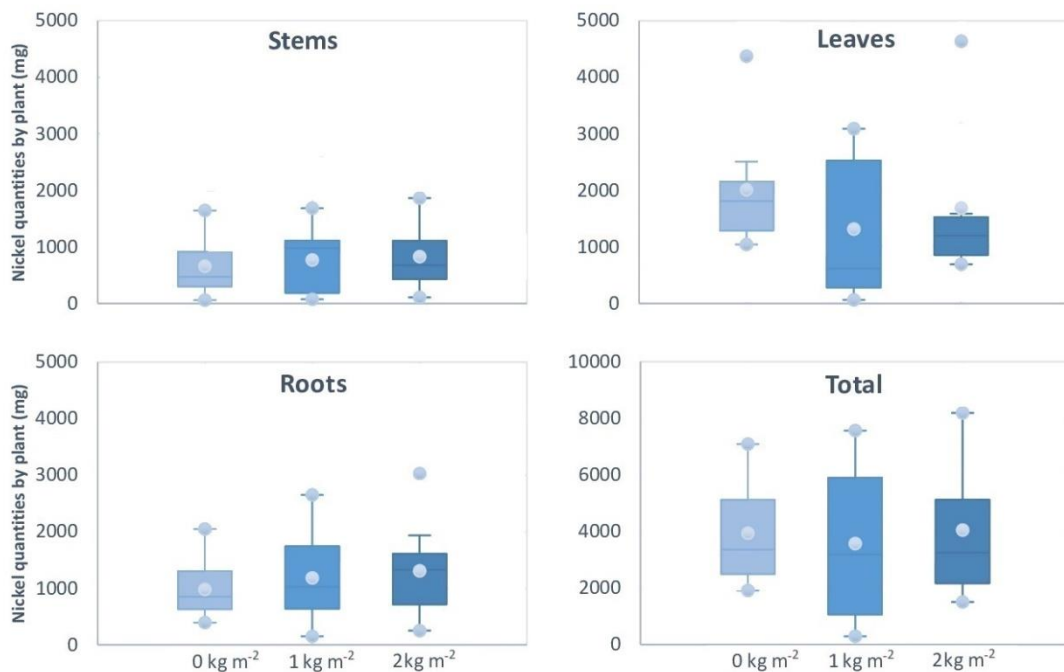


Figure 27 Nickel quantities of the different organs of *P. rufuschaneyi* in the organic treatments in mg per plant ($n=12$).

Plant harvest by coppicing was carried out every four months just before the OM treatments were applied (Figure 28). Coppicing had significant positive effects on the number of branches of *P. rufuschaneyi* in the OM treatments. The amounts of biomass harvested increased significantly with time, reaching 900 kg ha⁻¹ after 11 months of treatment, based on a density of two plants per square meter. No statistical variation in biomass production among OM treatments and control was observed ($0.14 < p < 0.94$). Therefore, no variation in Ni yield among these treatments was measured. With an average concentration of 1.6 wt% of Ni in the harvested biomass, an average Ni yield of 14.9 kg ha⁻¹ has been measured after approximately 20 months of cropping for a density of two plants per square meter (Figure 28). A Ni yield of upto 18.3 kg ha⁻¹ can be expected in the first year of harvesting, and a Ni yield of 37 kg ha⁻¹ yr⁻¹ can be extrapolated for the subsequent years. The most productive plants (~25%) had a yield of 55–115 kg Ni ha⁻¹ yr⁻¹ for a density of 2 plants per m² and a yield of 110–230 kg Ni ha⁻¹ yr⁻¹ for a density of 4 per m².

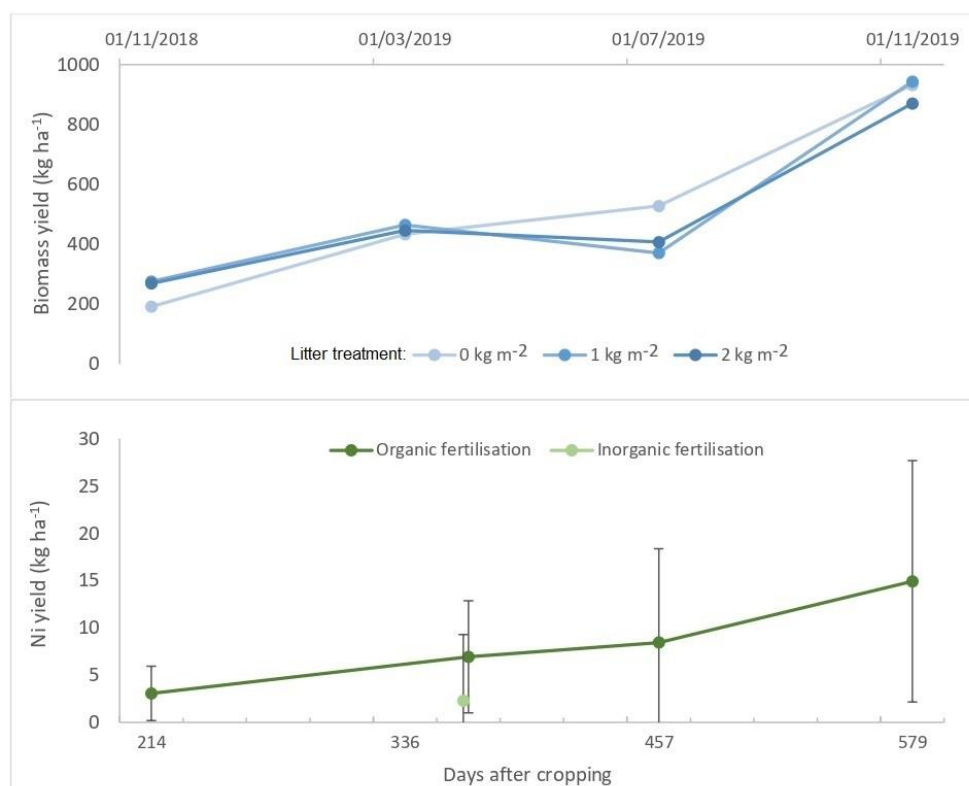


Figure 28 Harvested biomass overtime in the organic matter treatments in kg ha⁻¹ and Ni yield over time in the organic matter and inorganic fertiliser treatments in kg ha⁻¹

Only one harvest was obtained in in the eleventh month for the inorganic fertilisation trial due to their slower growth compared to the other trial. No difference in biomass production between inorganic fertilisation treatments and control was found ($p = 0.49$). The average amount of biomass was only 212 kg ha⁻¹, four-fold lower than the 2 kg OM m⁻² treatment.

D. DISCUSSION

The inherent extreme soil properties of ultramafic soils present unique challenges to the biomass production and the Ni yield of potential ‘metal crops’ when undertaking agromining in ultramafic soils. However, appropriate agronomic systems can be developed to maximise Ni yield (Nkrumah et al. 2018d). High DTPA-extractable Ni concentration is always a desirable property of soils for agromining operations (Nkrumah et al. 2016a). The organic matter amendment used in the present study significantly increased DTPA-extractable Ni concentrations in the surface soils ($p < 0.001$). The 1 kg m^{-2} treatment mimics the natural biogeochemical cycling in a pure stand of *P. rufuschaneyi*. The 2 kg m^{-2} treatment allows to observe a situation in which litter and Ni fluxes back to the topsoil are exaggerated and the evolution of DTPA-extractable Ni during the experiment shows that hyperaccumulator plants are responsible for a significant part of the available Ni in ultramafic topsoil as suggested in earlier works (Estrade et al. 2015; Echevarria 2018; Zelano et al. 2020). We stress that we employed non-traditional OM amendment, comprising of shredded *P. rufuschaneyi* which has inherent high Ni concentrations (Table 12). We consider that the increase of DTPA-extractable Ni is coming from the amended litter itself because of its inherent Ni-rich composition. Nevertheless, litter degradation can strongly modify soil parameters and could potentially increase or decrease Ni bioavailability. Conventional OM application to ultramafic soils reduce DTPA-extractable Ni concentrations, which may result in decreased or increased Ni yield depending on shoot Ni concentrations (Bani and Echevarria 2019; Broadhurst and Chaney 2016; Kidd et al. 2018; Nkrumah et al. 2019c). The OM amendments in this study did not appear to influence Ni yield, one year after application, despite the strong increase until saturation, a doubling, in the DTPA-extractable Ni concentrations in surface soils. The DTPA-Ni concentrations of the rhizosphere soils were, however, statistically lower than that of the surface soils for each of the OM treatment. Several questions arise: i) is the Ni enrichment due to OM application only limited at the surface soils? ii) how long will it take for the Ni in the litter to be released into the rhizosphere soils? iii) is enhanced plant Ni uptake responsible for the relatively lower DTPA-extractable Ni concentrations in the rhizosphere soils? iv) how does Ni from Ni-rich plant litter replenish the labile pool and influence the Ni biogeochemical cycle? It is clear that the short-term effects of the OM used in this study on biomass production and Ni yield are not apparent and more time would probably be needed to observe significant effects of the sharp increase in DTPA-extractable Ni. This implies that to fully realise the benefits of OM amendments on biomass production and Ni yield in tropical agromining ‘metal crops’ in

the short term, the OM treatments should be incorporated into the substrates rather than surface application. If the latter mode of application is employed, then the field must be prepared at least 12 months prior to planting. Litter degradation modifies the Ni cycle and it is still being monitored to assess the impact of the litter in a more significant timeline. Moreover, detailed Ni biogeochemical recycling investigations in the native habitats of tropical hyperaccumulator plants may provide insights on the long-term influence of Ni-rich litter fall on local labile Ni pools in a natural setting.

The present study confirms that *P. rufuschaneyi* responds negatively to N addition (urea), in terms of growth and Ni yield. Previous studies on *P. rufuschaneyi* reported that plant height, stem thickness and leaf count increased with a low N addition but decreased with a high N addition during a pot experiment (Nkrumah et al. 2019b). Moreover, application of NPK fertiliser had no statistical effect on plant growth and shoot Ni concentrations of *P. rufuschaneyi*. On the contrary, inorganic fertilisation significantly increases Ni yield of temperate Ni ‘metal crop’, *O. chalcidica* (Alvarez-López et al. 2016; Bani et al. 2007, 2015a, Broadhurst and Chaney 2016). NPK fertilisation had a positive effect on K bioavailability in soil, but no effect on leaf nutrient composition (Supplementary Data 1). This implies that only moderate NPK fertiliser application should be sufficient for tropical Ni agromining, using *P. rufuschaneyi* to replenish nutrients depletion due to plant harvesting as an exact compensation of the losses.

Nickel extraction varied from 3.1 to 14.9 kg Ni per ha⁻¹ per harvesting with an average of 37 kg Ni ha⁻¹ yr⁻¹, considering a theoretical density of 2 plants per m² and a mean concentration of 1.6 wt% Ni in shoots of *P. rufuschaneyi* (Figure 28). In considering the same density as inorganic treatments (4 plant per m²), an average Ni yield of 74 kg Ni ha⁻¹ yr⁻¹ is expected in the organic treatment. The most productive plants (~25%) had a yield of 110–230 kg Ni ha⁻¹ yr⁻¹ (4 plant per m²). Thus, the potential Ni yield of *P. rufuschaneyi* could be higher than the temperate *O. chalcidica* for which a maximum yield of 110–145 kg Ni ha⁻¹ yr⁻¹ is attained (Bani and Echevarria 2019). Although the Ni yields resulting from this study are lower compared to those reported by Nkrumah et al. (2019a) (250 kg ha⁻¹ yr⁻¹ with a density of 5 plants per m²), they could reach a mean of 94 kg Ni ha⁻¹ yr⁻¹ using the same density. Nevertheless, the amount of Ni extracted could decrease with an increase in density due to a possible competition between plants at higher densities. Future studies should test optimum plant densities for optimum agromining yields. For the inorganic treatment, Ni yield was relatively lower compared to the

OM treatments with an average of 3.4 kg Ni ha⁻¹ at harvest, translating to ~10 kg Ni ha⁻¹ yr⁻¹ (Figure 28). It is not clear whether inorganic fertilisation may enhance Ni yield in the long run. Overall, the time and mode of fertiliser application must be tested prior to field usage.

In addition to the treatments, other factors that may affect Ni yield include genetic variability in saplings, phenology, harvesting method and/or schedule, initial DTPA-extractable Ni and climatic conditions. In this study, flowering occurred mainly in June and September for each treatment (Figure 25). However, no differences of Ni concentrations were observed between the dates of harvesting for the OM treatments. Therefore, the flowering period seems not to affect Ni yield, contrary to the temperate hyperaccumulators *O. chalcidica* (Bani et al. 2015) and *O. lesbiacum* (Adamidis et al. 2017). Notably, seeds production is limited and may be related to the presence of *Epicephala* moths (Bouman et al. 2018). In the field, seed production may be between May and October, and therefore, this period may not be suitable for harvesting if seeds are required for propagation. Other innovative pollination approaches need to be considered to achieve high seed production for mass propagation. Mass propagation via cuttings is also a suitable propagation option, and its feasibility in the field needs to be tested. These mass propagation approaches could then be followed by plant breeding to select suitable cultivars for enhanced Ni yield.

The observations in this study suggest that coppicing is an important harvesting method for tropical ‘metal crops’ as it substantially increases biomass production and ultimately Ni yield. Future studies should probably find the optimal coppicing application (how many applications per time) in order to get the highest biomass production and/or highest Ni yield. The findings in this study suggest that DTPA-extractable Ni concentrations higher than 160 mg kg⁻¹ may produce optimum Ni yields when employing *P. rufuschaneyi* on Cambisol. More research is required on Ni uptake by plants to develop predictive tools (Nkrumah et al. 2016a; van der Ent et al. 2019). Precipitation has strong effects on the growth of *P. rufuschaneyi* and the effect of soil moisture on Ni yield needs further research.

This study evaluates the agronomic systems required to develop viable ‘real-life’ tropical agromining using *P. rufuschaneyi*. Organic matter and inorganic fertiliser did not improve biomass production and Ni yield in the short term. Nickel yield was 74 kg ha⁻¹ yr⁻¹ for the more productive plot, with a density of 4 plants per m². Through a selection of the most productive plants, Ni yield could vary from 110–230 kg ha⁻¹ and may increase significantly with

appropriate plant breeding and agronomic systems. Coppicing has strong effects on biomass production and overall Ni yield and may be an important harvesting method for tropical ‘metal crops’. The field trials are still ongoing, and the effects of inorganic and OM treatments may be significant in the long-term.

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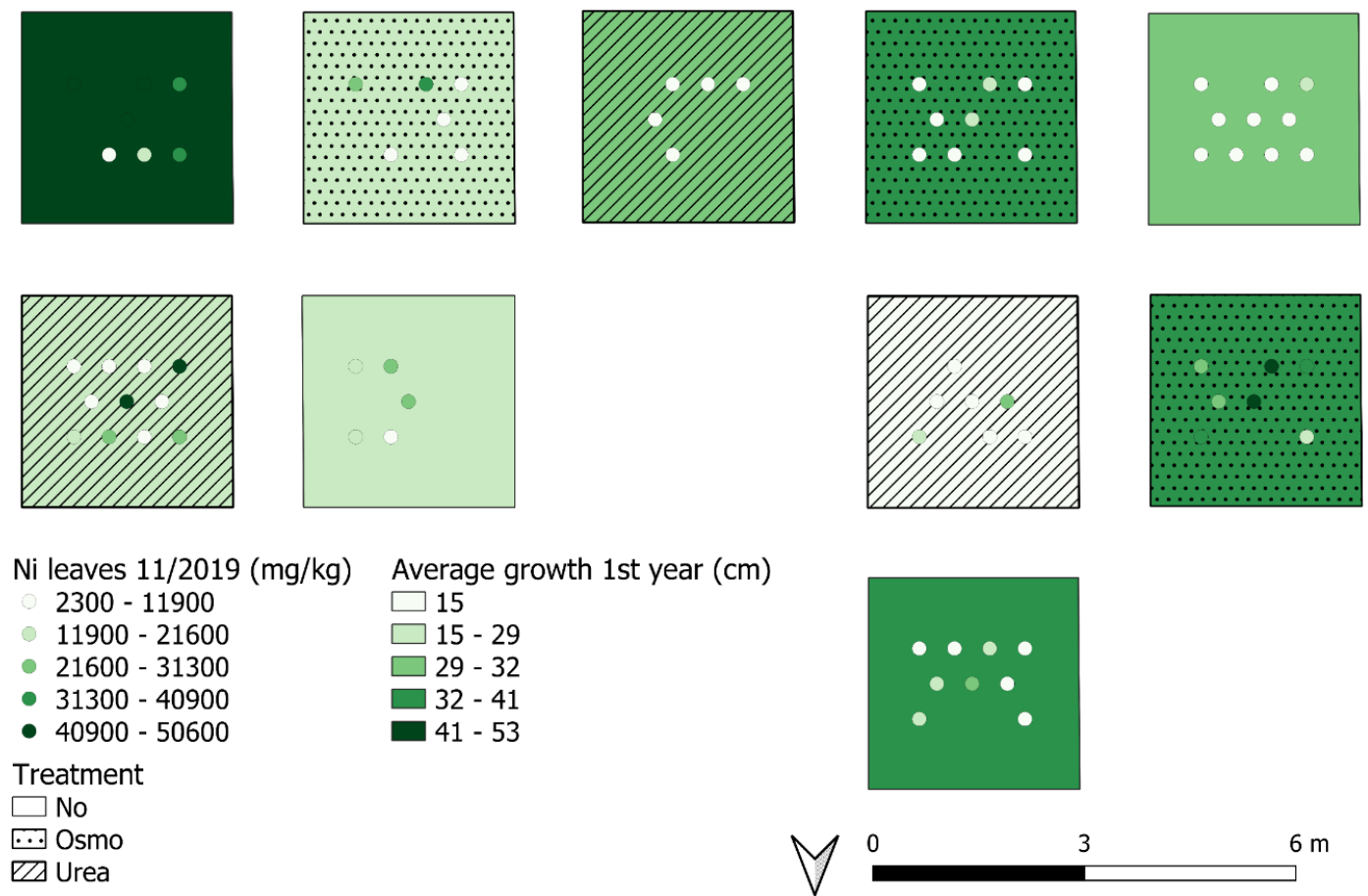
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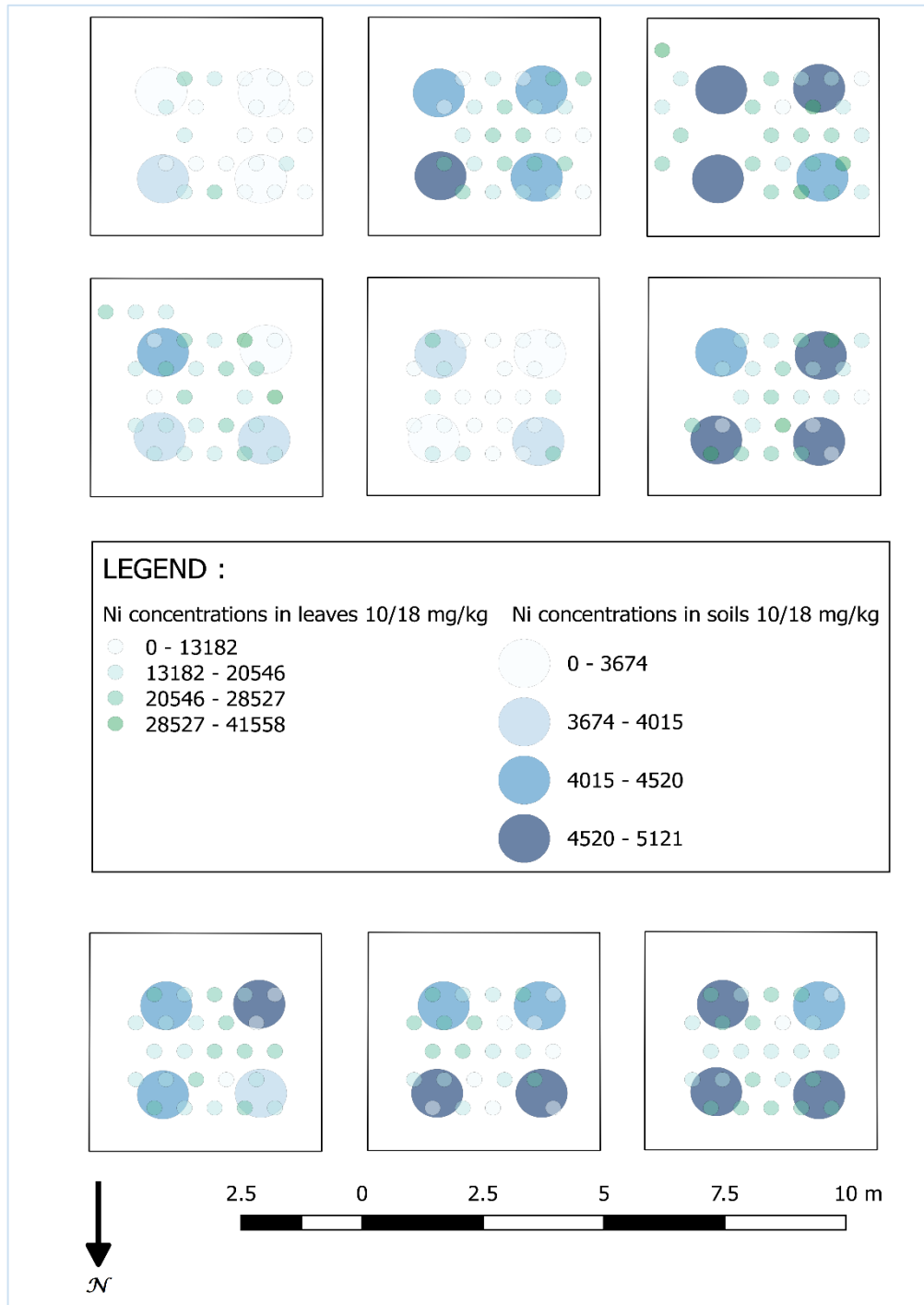
Supplementary Table 9.:Element concentrations in soils (n=9) and in the leaves (n=33) of *Phyllanthus rufuschaneyi* of the plants amended with inorganic fertilisation in January 2020. The elemental concentrations of organic and inorganics treatments are given in mg kg⁻¹.

		Leaves							DTPA		CEC			Polisen
		Ca	K	Mg	Ni	P	S	Zn	Ni	Mn	Ca	K	Mg	
Control	maximum		18 000	7000	50 000	1800	1000	430	150	90				10
	minimum		2000	1000	2500	500	8000	30	90	30				4.0
	average	9100	4500	3400	16 700	1000	2400	150	120	40	3500	45	1500	6.4
NPK	maximum		8900	1500	45 000	2100	1200	340	170	100				10
	minimum		2000	8000	3400	500	6000	30	120	60				6.7
	average	9000	4300	4000	18 400	1100	2500	150	140	70	2900	62	1400	8.3
Urea	maximum		14 000	1900	46 000	2400	1400	30	160	70				8.0
	minimum		1600	8100	2300	700	5000	330	70	40				5.4
	average	9200	4200	4100	12 600	1200	2400	120	130	50	3600	49	1600	6.7

Supplementary Figure 8.: Ni concentration in leaves (mg kg^{-1}) and average growth (cm) of *Phyllanthus rufuschaneyi* amended with chemical fertilisers.



Supplementary Figure 9.: Ni concentration in leaves (mg kg^{-1}) and average growth (cm) of *Phyllanthus rufuschaneyi* amended with organic fertilisers



GENERAL CONCLUSION AND PERSPECTIVES

Hyperaccumulator plants can accumulate high amounts of metals in their aerial biomass, making them unique and useful for green nature-based technologies. Agromining is a good example of these technologies, using hyperaccumulator plants to recover metals from mineralised soils. Nickel agromining is currently in the transition from pilot-scale to large-scale field demonstrations in some specific places in Europe (*i.e.* Albania, Spain, Greece), and South-eastern Asia (*i.e.* Malaysia). Tropical agromining is still understudied compared to temperate agromining, and its characteristics could drastically change from agromining in temperate regions, especially because it uses perennial woody species and not herbs. Nickel yield in Southeast Asia could potentially reach 250 kg ha⁻¹ of Ni per year, so more than in temperate regions (Nkrumah et al. 2019a).

The main objective of this study was to understand and assess the biogeochemical cycling of Ni in a tropical hypernickelophore stand in both its natural environment and under agromining cropping conditions. Litter amendments were carried out at Pahu “metal farm”. The first rate to be applied, consisting of 1 kg litter m⁻², was similar to the amounts falling on the topsoil of *P. rufuschaneyi* in its natural habitat. The higher dose corresponds to twice as much as the litter amount in a natural hyperaccumulator forest (2 kg litter m⁻²). Finally, a treatment with no other input than the decaying leaves from the non-harvested standing biomass was included in the experiment to achieve a full sensitivity study of the role of litter return to the Ni cycle. Furthermore, we were also interested in how Ni yield (biomass production and Ni accumulation in shoots) could be improved by nutrient status in soils and fertilisation (NPK and Urea).

A. ELEMENT and Ni BEHAVIOURS

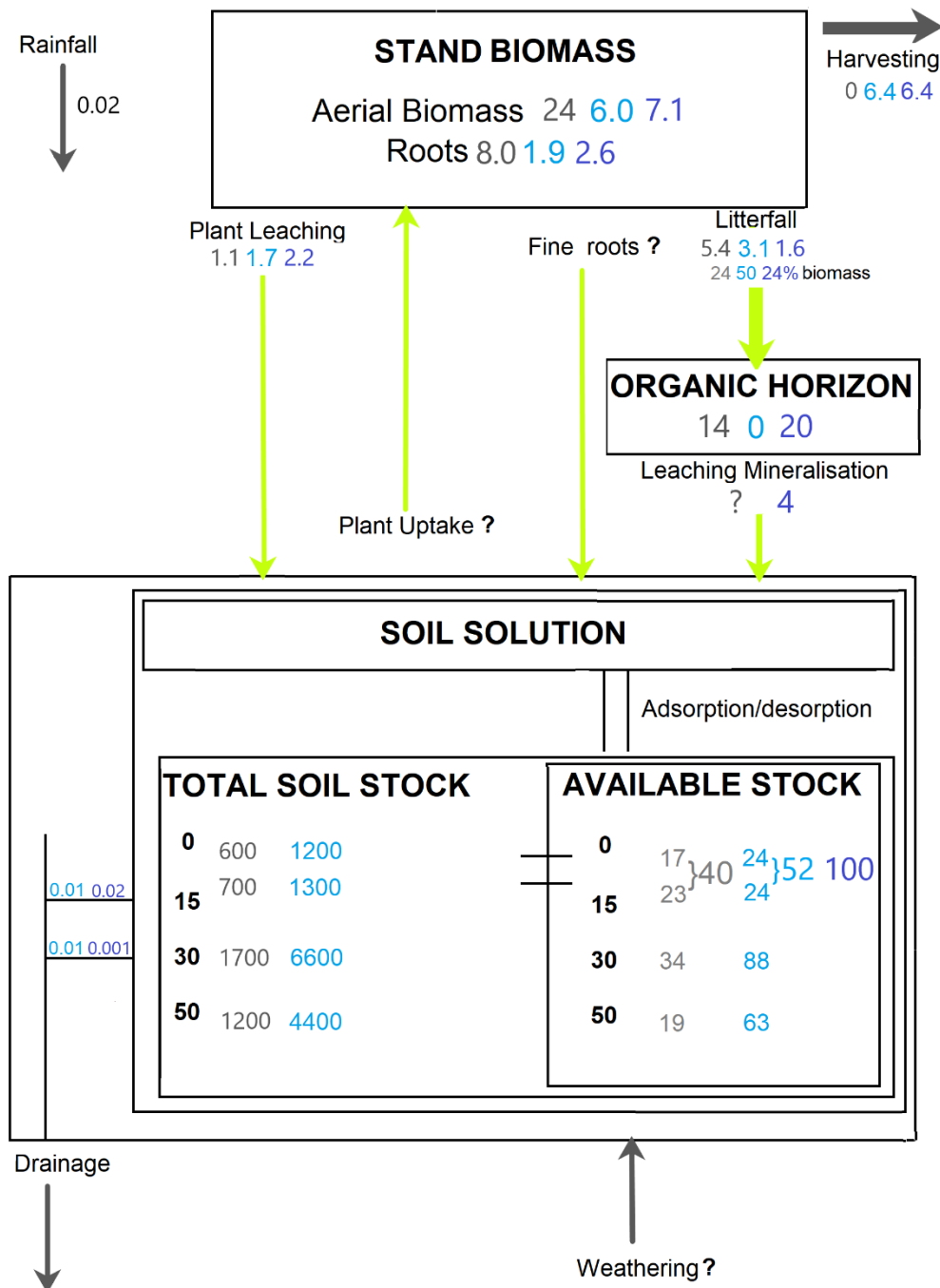
a. Biological and biochemical cycles

The biological cycle of *Phyllanthus rufuschaneyi* was studied through the study of element concentrations in standing biomass and litterfall. Ni was slightly more concentrated in old leaves than in young leaves and tended to be more concentrated in leaf litterfall than in standing leaves. These could be due to a **limited re-uptake of Ni from senescent leaves to functional leaves with a phloem-driven building up of Ni concentrations in leaves.**

The reabsorption of elements was different between cropped and wild *P. rufuschaneyi*. In the forest, the trees did not reabsorb Ca and Mn as they did in the cropping system. The K and Mn annual budget could be negative at Pahu since throughfall flux was overestimated due to the unknown soil cover of *P. rufuschaneyi*. This non-positive budget can explain the change in these nutrients biological cycles. This reabsorption could be explained by different interactions

between nutrients in soils or by the impact of harvesting on various element stocks. Preferential reabsorption of these element could be due to a decrease in their available concentrations in soil due to root depletion under sustained harvesting.

Figure 29 Nickel biogeochemical cycle of *Phyllanthus rufuschaneyi* in the first tropical metal farm and in an endemic forest at Garras in 2019. Stocks in $g\ m^{-2}$ and fluxes in $g\ m^{-2}\ y^{-1}$. (Forest = grey; Metal farm: 0 $kg\ m^{-2}$ of litter = light blue; 2 $kg\ m^{-2}$ of litter = dark blue).



Main nutrients inputs are litterfall and throughfall for biochemical sub-cycle. The rate of litterfall in this hyperaccumulator forest was comparable to the litterfall of numerous other tropical humid forests ($1.1\ kg\ m^{-2}\ y^{-1}$) (Bray JR and Gorham 1964, Burghouts et al. 1992, 1998).

The major part of litterfall tended to occur between mid-February and early April 2019, which corresponds to the driest period in the Mount Kinabalu area. The quantities of the elements contained by the leaf litter of hyperaccumulator plants did not differ from other tropical forests (e.g. Proctor 1983), except for Ni.

Litterfall is the major flux of Ni input in natural hyperaccumulator forests (77%; 5.4 g m⁻² y⁻¹) and in cropping system (3.1 g m⁻² y⁻¹), besides mineral weathering which was not studied here (Figure 29). Nickel litterfall concentration of plant treated with a high or low dose of litter was statistically equal to the Ni concentration of the hyperaccumulator forest litterfall. For a plant growing on soils that were not treated with litter, litterfall corresponded to 50% of its aerial biomass and was 50% more concentrated in Ni than for the other plants from litter-treated plots. For treated plants, litterfall corresponded to 20–25% of their aerial biomass, and for the endemic forest, litterfall also corresponded to 20–25% of the aerial biomass (Figure 29). **Harvesting seems to have no impact on Ni return by litterfall contrary to litter decaying.** Nevertheless, the conditions and the age of the plants are not the same between the two sites. *Phyllanthus rufuschaneyi* grows naturally mostly under shade in the secondary forest as only the oldest trees (7–8 years old) form the canopy, whereas in the agromining experimental site all plants have the same age (6mo–1yr old at plantation) and grow under full light conditions. This change in exposure to light could also have an impact on the litterfall biomass. Setting up litter traps under non-harvested plants at a planted site such as in Pahu, outside the plots of the experimental design, could help to answer this question.

The rate of leaf litter degradation measured in the agromining tropical farm is the same as the rate of degradation of leaf litter in the standing forest of *P. rufuschaneyi* (k=1.1). After almost two years of cropping, microorganisms can degrade litter as rapidly as in the natural hyperaccumulator forest. Because of the small mesh size of the litter bags which were used, it was evidenced that the effect of macroinvertebrate on *P. rufuschaneyi* was low in both sites. Nickel turnover was high in leaf litter in both sites even if wood degradation is a rather slow process (k=0.3). Nevertheless, litter degradation from hyperaccumulator biomass in temperate ecosystems seems to be faster (e.g. Echevarria et al. 2021).

Ni Stocks balances

In the hyperaccumulator natural forest, **no significant increase of total Ni concentration and stock in the upper horizon was visible, despite a significant litterfall rate** (11 kg m⁻²). At the first tropical metal farm, after 15 months of litter treatment, no

significant difference in Ni availability between the low dose and the high dose treatment were visible but both were higher than the treatment without litter. Taking into account that the low-dose treatment corresponds to the litter amount in the Ni hyperaccumulator forest in July 2018; litter degradation increased Ni availability in both environments, but this increase was limited, since after 15 months of treatment Ni-DTPA concentration was the same for the two litter treatments. At Pahu site we could follow free water drained to the lysimeters. Soils amended with litter tended to leach more Ni below 15cm than non-amended soils although it was not significant ($p=0.11$). However at 30 cm, in the B horizon, Ni concentration in drained water was many times lower than at 15 cm. Nickel loss from the upper horizons is logically stored in the B horizon (between 15 and 40). Over the long term, the B horizon could thus gain significant stocks of exchangeable Ni to *P. rufuschaneyi*, despite Ni in this horizon is less available than in the surface horizons originally. Long term changes in the available pool of Ni in the B horizon is a trend that should be verified in long-term monitoring of these plots.

After almost two years of cropping, no Ni DTPA decrease was noticed on any of the treated plots, including the no-return treatment. Ni availability tended even to increase after cropping with a faster rate for the double dose than the single dose treatment. However, they seemed to reach a similar plateau after two years whatever the litter input rate indicating a sort of saturation of Ni exchangeable sites. **The stability of the Ni available pool could be due partly to the Ni return by litterfall.** Even under agromining, litterfall cannot be neglected as Ni return corresponds to 25–50% of the Ni output by harvesting (*i.e.* in this case, $65 \text{ kg ha}^{-1} \text{ yr}^{-1}$). In addition to the A horizon composition, B horizon composition could be a key factor for Ni agromining: the release of Ni from this significant stock could have an impact in long term agromining operations and it actively stores the soluble Ni released over time. It must have an active role also in feeding the root systems with Ni that is returned to the surface. The other return that could influence this pool is the weathering of primary minerals from the bedrock (C horizons and stones). We did not measure this flux in this study, but it is crucial for a complete understanding of the full loop. It should be targeted in future research. Further experiments could be carried out at different spots of the first tropical metal farm because the composition of the bedrock and its susceptibility to weathering can vary enormously (even on the same site). Indicators at the surface, other than Ni DTPA, could be described to give a better idea of Ni weathering. It is essential to establish tropical hyperaccumulator crops on soils with a sufficient reservoir of available Ni.

Ni hyperaccumulator plant role

The role of Ni hyperaccumulator plants in tropical ultramafic ecosystems can be better understood after these first results, in particular after the careful study of the effect of the recycling of their Ni-rich and nutrient-rich biomass to the soil as well as the effect of the quantity and quality of water that leaches the shoots of hyperaccumulators before returning to the soil. Firstly, a plant cover composed of mostly hyperaccumulators will induce a fast and strong recycling of Ni in the available pools of the entire soil profile. Secondly, hyperaccumulators seem to re-saturate completely the storage capacity for available Ni of the entire surface horizon. Saturation is reached faster when litter returns are higher. In the case of Pahu plots, we can estimate this capacity to be of 400 mg kg^{-1} , whereas the initial value was 200 mg kg^{-1} before the experiment started. These findings are quite consistent with what was reported from stable isotope discrimination studies of ultramafic soils and Ni hyperaccumulator plants (Estrade et al. 2015; Zelano et al. 2020). It seems that the strong K recycling observed in these systems is also of high importance in understanding the ecological role of hyperaccumulator plant species. Nickel hyperaccumulator plants strongly recycle these two elements in the topsoils, but also in the available pools of B horizons thus creating suitable conditions for other plant species. Whereas Ni can be seen as an allelopathic agent, K is rather favourable for other plant species that would suffer from its scarcity in ultramafic soils. It is amazing to note that, despite strong accumulation and recycling of K achieved by *P. rufuschaneyi*, the species does not tolerate high K fertility in soils (Nkrumah et al. 2019c). It is also not tolerant to high organic matter contents in soils. It is therefore clear that *P. rufuschaneyi* is a pioneer species that comes first in the ecological succession on low altitude ultramafic cambisols of the Kinabalu area in Sabah. **Its strong capacity to spread from sexual reproduction (flowering can occur in only 6 months from germination), its strong tolerance to full sunlight and its biogeochemical behaviour (Bouman et al. 2018) clearly advocate for its role as a true ruderal and pioneer species whose role is to increase soil chemical and biological fertility after the soil has been cleared from vegetation (landslide, fire).** The only aspect that is poorly understood is **what role does the concentration of Ni available pools in topsoils play in this ecological succession?** It might be that Ni is tolerated by most of the endemic flora of these ultramafic cambisols, some hyperaccumulators (e.g. *Rinorea* aff. *bengalensis*) which are shade trees might benefit from this Ni build-up in soils being a second-stage vegetation in the ecological succession.

B. IMPROVING Ni AGROMINING

Not all ultramafic soils can support tropical nickel agromining (Nkrumah 2018). **The improvement of Agromining can be done by numerous ways.** In this project, synthetic and organic fertilisation were tested.

During this study, harvesting at different heights was tested; coppicing at 50 cm seemed to be preferable for sustained harvesting. Harvesting on a more frequent pace than annual is not recommended due to overall lower biomass production. Harvesting increased the branches count and leaf biomass. Fast harvesting is recommendable before plants reach 50 cm. Furthermore, *P. rufuschaneyi* is resistant to common pesticides, but their use must be avoided.

NPK fertilisation can enhance hyperaccumulator plants' growth in temperate regions (Bani et al. 2015a, b). Our experiments demonstrated no effect of NPK addition on Ni yield for a dose of 370 kg ha⁻¹. Thus, fertilisation did not change the plants' behaviour, except for N. Urea addition at a dose of 120 kg ha⁻¹ decreased leaf Ni concentration, as already shown in pots by (Nkrumah 2019b, c). Ni is needed as N for urease activity in plants; this enzyme catalyzes the hydrolysis of urea. Increase of N concentrations could maybe decrease the requirement of hyperaccumulators plants in Ni due to a lower urease activity. Furthermore, no effect on soil pH of treatments was detected. **No fertilisation with nutrients is therefore really needed to improve Ni yield of *Phyllanthus rufuschaneyi*, at least after two years of culture.** It might become needed to balance fluxes over the long term.

Organic amendments can enhance the growth of hyperaccumulator plants in temperate regions (Álvarez-López et al. 2016). In this study, we used non-conventional organic amendments. Crushed aerial biomass of *P. rufuschaneyi* itself was spread on the soil at two doses. These treatments were applied from November 2018 to November 2019. After 15 months of treatments, **organic amendments did not affect Ni biomass nor leaf Ni concentrations, even if Ni DTPA in soils increased.** However, the overall plant biomass in the 2 kg OM m⁻² treatment tended to be higher than that of the non-treated plots (p = 0.16).

Organic and inorganic treatments were tested in two different zones of the first tropical metal farm with different Ni DTPA contents in topsoil. Inorganic fertilisation was applied on plant growing on soil with an available Ni concentration lower (120 mg kg^{-1}) than plant fertilised with organic matter (160 mg kg^{-1}). Ni yield from these two plots was completely different, even for the same NPK treatment (370 kg ha^{-1}). Ni yield of organic control was seven times higher (74 kg m^{-2}) than the Ni yield of NPK treatment of inorganic amendment (10 kg m^{-2}) for the same density (4 plants m^{-2}). We hypothesize that this lower Ni yield was due to a lower Ni availability. **Ni availability of 160 mg kg^{-1} of Ni would be the minimum to have an economical tropical agromining with *P. rufuschaneyi*.** This difference could be partly due to a difference of Ni total concentration, Ni-bearing phase composition and soil texture. The soil of the inorganic treatment tended to have a more clayey texture. Further studies of soil composition variations across the experimental field of Pahu (Nkrumah et al. 2019a, d) could help to understand this effect.

C. Tropical agromining PERSPECTIVES

Numerous perspectives exist to improve tropical Agromining. Agronomic practices have to be adapted at each Ni hyperaccumulator cultivated. **Ni yield obtained in this study ($65\text{-}75 \text{ kg ha}^{-1}$) is just a preliminary study (the purpose of this study was not to reach the highest yield possible), Ni yield of tropical Agromining could be sustainably increased.** Co-cropping with legumes can increase Ni yield in temperate regions (Saad et al. 2018) and could improve tropical agromining too. Since the beginning of 2020, co-cropping between *P. rufuschaneyi* and herbaceous edible legumes is tested in Pahu field to measure the potential of this technique. Legumes are interesting for their capacity to increase soil nitrogen content, even if the chemical addition of this element decreases Ni concentration in plants in many cases. Of course, no NPK fertilization would be added in this case and the system would only get N from the legumes. Density tests are also currently being carried out in the first tropical metal farm to assess the best density over the long term. Testing agroforestry practices in future cropping experiments would be desirable. Furthermore, installation of a drainage and water system to improve biomass growth during the dryer season (February and March) could be put in place to test the effect in increasing Ni yield.

Not all the fluxes of Ni were measured in this study. Some significant fluxes of Ni were maybe neglected. **Rock weathering fluxes and inputs to the soils could be a critical flux to understand Ni behaviour in these environments.** Its calculation is needed to understand the long term behaviour of tropical hypernickelophores in natural and anthropic systems. Weathering could influence the extern Ni biogeochemical cycles strongly. Furthermore, roots uptake and degradation could influence the Ni and nutrients intern cycles and need to be studied too. Modelling of the different fluxes measured and the weathering flux, as well as deeper texture studies, would help to confirm the longevity of tropical agromining and the budget of available stocks.

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TRADUCTION française

A. INTRODUCTION

Les roches ultramafiques, qui sont originaires des dorsales océaniques, sont principalement composées de minéraux mafiques et de minéraux ferromagnésiens (Brooks 1987 ; Guillot et Hattori 2013). Les roches et sols ultramafiques sont enrichies en Ni et couvrent plus de 3 % de la surface de la Terre (Guillot et Hattori 2013). Les régions ultramafiques sont des zones d'intérêt pour l'implantation de mines de Ni, comme en Nouvelle-Calédonie ou en Indonésie. Les mines à ciel ouvert de minerai de nickel ont un impact négatif sur la biodiversité de ces régions, notamment par la destruction des sols et de la couverture végétale. La flore de ces régions est très particulière en raison de l'environnement édaphique spécifique, avec des concentrations toxiques de Mg et de Ni, et un manque de nutriments (Proctor et al. 1999). L'adaptation des plantes aux sols ultramafiques représente un système précieux et expérimentalement traçable pour les écologistes et les généticiens (Brady et al. 2005).

En effet, certaines plantes endémiques des sols ultramafiques ont développé des mécanismes écophysiologiques pour accumuler des concentrations extrêmes d'éléments traces ; elles sont connues sous le nom de plantes hyperaccumulatrices (Reeves 2003 ; van der Ent et al. 2013). A Sabah, 31 espèces de plantes hyperaccumulatrices de Ni sont actuellement connues et se situent principalement sur les Cambisols Hypermagnésiques (van der Ent et al. 2016a, b, 2019). L'ordre des angiospermes (Malpighiales) est particulièrement intéressant pour la recherche de nouveaux taxons en raison de la découverte de nombreuses espèces de plantes hyperaccumulatrices dans cet ordre qui pourraient avoir des applications dans le domaine de la phytomine (Reeves 2003). Un grand nombre de plantes hyperaccumulatrices de Ni appartiennent à cet ordre (Malpighiales), qui à son tour, appartient au clade COM (Jaffré et al. 2013 ; Reeves et al. 2017). Cet ordre contient également la famille des Phyllanthacées qui compte le plus grand nombre de plantes hyperaccumulatrices de Ni, comme à Cuba et en Nouvelle-Calédonie (Reeves 2003 ; Jaffré et al. 2013).

Au cours des dernières décennies, la demande mondiale de métaux stratégiques a augmenté et continuera d'augmenter, notamment pour le Ni. De nouvelles méthodes d'extraction des métaux sont apparues, elles pourraient remettre en question les techniques d'extraction traditionnelles. L'agromine en est une. Il s'agit d'une chaîne de processus où des plantes hyperaccumulatrices de métaux sont cultivées et récoltées pour la récupération des minerais. L'objectif de cette technologie est de produire des métaux à un coût environnemental

relativement faible et éventuellement à un coût économique tout aussi faible, suffisant pour concurrencer les techniques minières traditionnelles. Cette méthode de culture peut être développée sur des sols naturellement enrichis en métaux, tels que les sols ultramafiques ou sur des déchets miniers dont l'extraction par des méthodes métallurgiques conventionnelles n'est pas viable économiquement. Ce procédé est actuellement étudié dans les régions tempérées avec des résultats très prometteurs. Par exemple, un rendement de $145 \text{ kg ha}^{-1} \text{ y}^{-1}$ de Ni a été obtenu en Albanie en utilisant l'hyperaccumulateur de Ni *Alyssum murale* (Bani et Echevarria 2019). Dans les tropiques, notamment en Malaisie, il existe d'importantes possibilités non réalisées d'exploitation économique du Ni (Nkrumah et al. 2021).

L'étude de la dynamique des gisements de nickel dans les sols ultramafiques et leur quantification sont essentielles pour déterminer la durée et la viabilité potentielles de l'exploitation agricole du nickel. Les réserves de Ni disponibles dans les sols seront fortement épuisées par l'absorption continue par les hyperaccumulateurs et leur récolte. Des études récentes ont démontré que les plantes hyperaccumulatrices influencent directement les réserves en Ni disponible et total dans les horizons supérieurs des sols ultramafiques (Estrade et al. 2015). Les plantes hyperaccumulatrices de Ni pourraient jouer un rôle majeur dans le maintien des concentrations élevées en Ni à la surface des sols pendant l'altération et la pédogenèse (Echevarria 2018).

Pour être utilisé en Agromine, les plantes hyperaccumulatrices doivent accumuler du Ni à des concentrations élevées, comme les "hypernickelophores" qui accumulent du Ni à des concentrations supérieures à 1% en poids. Ces plantes doivent être faciles à cultiver, avoir une croissance raisonnablement rapide et produire une grande quantité de biomasse. *Phyllanthus rufuschaneyi*, une espèce nouvellement décrite, est connue pour être limitée à seulement deux localités à Sabah (c'est-à-dire Bukit Hampuan et Lompoyou Hill), ce qui rend cette plante extrêmement rare (Bouman et al. 2018). Le type de sol dans son habitat d'origine est un jeune Cambisol hypermagnésien (érodé) sur un substrat rocheux fortement serpentinisé (van der Ent et al. 2018). Cette plante accumule des concentrations exceptionnelles de Ni dans ses pousses, avec jusqu'à 2,5 % en poids dans les vieilles feuilles et 1,2 % en poids dans les rameaux (van der Ent et al. 2015 ; van der Ent et Mulligan 2015 ; Bouman et al. 2018). Les concentrations de Ni dans le phloème et dans les graines sont également très élevées, avec des concentrations moyennes de 0.9% et 1.8% (van der Ent et Mulligan 2015).

Compte tenu des concentrations élevées de Ni dans les pousses, de ses nombreuses tiges, de sa repousse rapide après le taillis, de son besoin de lumière et de sa résistance aux ravageurs, *P.*

rufuschaneyi est un candidat idéal pour la "culture de métaux" pour l'Agromine tropicale du Ni (Bouman et al. 2018 ; Nkrumah et al. 2019a). C'est pourquoi cette espèce tropicale d'hypernickelophore a été sélectionnée pour être déployée dans des expérimentations de terrain à grande échelle en tant que meilleur candidat à Sabah en Malaisie (Nkrumah et al. 2019d) et a été choisie comme espèce pour ce doctorat visant à évaluer l'impact de l'hyperaccumulation dans le cycle biogéochimique du Ni dans les écosystèmes ultramafiques.

Néanmoins, les facteurs et les traitements pour augmenter la production de Ni précédemment étudiés en conditions tempérées (Bani et al. 2015 ; Nkrumah et al. 2016), peuvent ne pas être les mêmes sous les tropiques en raison des caractéristiques spécifiques des plantes hyperaccumulatrices tropicales. L'agromine des "cultures métalliques" tropicales n'avait pas été testée avant que Nkrumah et al. (2018a) étudie les conditions permettant d'optimiser la croissance et le développement de deux hypernickelophores à Sabah (Malaisie). La réponse de *Rinorea aff. bengalensis* et de *Phyllanthus rufuschaneyi* à l'amendement de différents éléments a été étudiée par des expériences en pots (Nkrumah et al. 2019b,c,d). Le rendement en Ni de *P. rufuschaneyi* diminue avec l'apport de N et de OM, contrairement à la réaction à l'ajout de Ca et de S. Le rendement en Ni de cet hypernickelophore tropical pourrait potentiellement atteindre 250 kg ha⁻¹ an⁻¹ (Nkrumah et al. 2019a). Des essais en plein champ dans des conditions tropicales contribueront à améliorer les performances de toute culture potentielle d'agromine. L'agro-industrie du nickel pourrait avoir un potentiel économique élevé, mais des démonstrations à grande échelle sont certainement nécessaires pour fournir des preuves convaincantes pour les opérations commerciales (Reeves, van der Ent, & Baker 2018b ; Nkrumah et al. 2019).

Les cycles biogéochimiques des plantes tropicales hyperaccumulatrices sont encore mal compris. La description de la dynamique du Ni au sein de l'écosystème des plantes hyperaccumulatrices est nécessaire pour élucider le rôle écologique des plantes hyperaccumulatrices dans leur environnement naturel, mais aussi pour comprendre leur comportement potentiel dans les systèmes d'agromine tropicale. La litière et la décomposition semblent affecter le retour du Ni dans le sol, mais l'étude des cycles interne et externe du Ni est nécessaire pour comprendre explicitement l'importance de la biomasse des hyperaccumulateurs et du cycle du Ni. L'exploitation agricole et l'exportation de la biomasse riche en Ni interrompra le cycle. Les plantes hyperaccumulatrices sont également capables d'exporter de grandes quantités de nutriments des sols (par exemple Ca K P et S). Il est donc important d'évaluer l'impact de la fertilité des sols dans des conditions de terrain à long terme sur le cycle de ces éléments et sur l'agronomie de ces plantes.

Pour résumer, les questions clés que ce doctorat abordera sont les suivantes :

- ⊗ Quels sont les cycles du Ni (interne et externe) et leur impact sur le fonctionnement écologique du forêt tropical d'hyperaccumulateurs ? Quel est le rôle des plantes hyperaccumulatrices dans la succession écologique sur les sols ultramafiques ?
- ⊗ Quelle est la vitesse des flux de Ni à travers les compartiments sol-plante et quelle est le renouvellement du Ni dans un système tropical d'hyperaccumulation ? À quelle vitesse une culture tropicale Agromine peut-elle épuiser le Ni dans le sol ?
- ⊗ Quels sont les impacts de la fertilisation inorganique et organique sur la durabilité de l'agromine du Ni en milieu tropical ?

L'objectif principal de ce doctorat est la caractérisation des cycles des métaux et des nutriments des plantes tropicales hyperaccumulatrices, à la fois dans une parcelle d'hyperaccumulateurs plantée et dans un peuplement naturel (forêt secondaire). En outre, de nombreuses autres questions fondamentales se posent, notamment le rôle de l'hyperaccumulation dans la pédogenèse ultramafique, et l'efficacité des cycles biogéochimique des nutriments et des métaux dans les peuplements de plantes hyperaccumulatrices.

De ces questions scientifiques découlent les objectifs suivants :

- ⊗ Étudier les cycles biogéochimiques d'une forêt naturelle de *P. rufuschaneyi* afin d'apprécier et d'évaluer les flux naturels de Ni dans l'écosystème ;
- ⊗ Manipuler un tel écosystème afin d'effectuer un test de sensibilité de l'écosystème pour le flux suivant : retour de la litière au sol ; de l'absence de retour (exportation) à un doublement du retour.
- ⊗ Sur la base de la compréhension du rôle du cycle biogéochimique litière/Ni dans l'alimentation de *P. rufuschaneyi* et des besoins en NPK et Ni de la plante, optimiser le système de culture de *P. rufuschaneyi* pour l'extraction du Ni.

Cette thèse est structurée en quatre chapitres afin d'aborder les trois objectifs. Le premier chapitre correspond à l'état de l'art, qui présente l'étape la plus récente de la recherche en agromine tropicale. Le comportement biogéochimique de la forêt naturelle d'hypernickelophore est décrit dans le deuxième chapitre, tandis que le comportement d'une parcelle d'agromine tropicale est présenté dans le chapitre trois. Le chapitre quatre est consacré à l'amélioration de l'Agromine tropicale par la fertilisation organique et chimique.

B. RÉSUMÉ des articles

a. Cycles biogéochimiques d'une forêt tropical d'hyperaccumulateurs de Ni

Le cycle biogéochimique du Ni par les plantes hyperaccumulatrices tropicales est inconnu, bien que l'on soupçonne qu'elles jouent un rôle majeur dans le recyclage de cet élément dans les écosystèmes ultramafiques. Dans cette étude, nous présentons le cycle biogéochimique du Ni (et d'autres éléments) par un hyperaccumulateur tropical de Ni, par *Phyllanthus rufuschaneyi*, qui est une des espèces les plus prometteuses au monde pour l'agromine. L'espèce a été étudiée dans une jeune forêt secondaire de l'État de Sabah (Malaisie) où elle est présente comme espèce dominante sur un Cambisol ultramafique. Pendant deux ans, nous avons surveillé une parcelle de 100 m² et recueilli des informations sur les conditions météorologiques, l'augmentation de la biomasse, la fertilité du sol, les flux d'eau vers le sol et les flux de litière pour différents éléments dont le Ni. Le cycle du Ni est principalement déterminé par les flux internes, notamment la dégradation et le recyclage de la litière riche en Ni. En près de deux ans et demi, le flux de nickel dans la litière correspond au stock total de nickel de la litière (5,2 g m⁻² an⁻¹). La rotation du Ni varie considérablement en fonction des différentes propriétés d'accumulation du couvert végétal, ce qui souligne l'influence extrême des hyperaccumulateurs de Ni dans la constitution des stocks de nickel disponibles dans les couches arables, comme on le soupçonnait déjà dans les systèmes ultramafiques tempérés. La litière et les pluviollessivats contribuent de manière substantielle au cycle du phosphore, du soufre et du potassium dans l'écosystème ; les pluviollessivats contribue respectivement à 2, 220 et 20 fois plus aux flux de nutriments du phosphore, du soufre et du potassium par rapport à la litière. Il convient de tenir compte de ces observations lors de la conception de l'agromine tropicale, car les stocks de Ni pourraient être plus labiles qu'on ne le pensait initialement, et la récolte de la biomasse riche en Ni et en nutriments pourrait potentiellement affecter le Ni disponible (et les nutriments) pour la prochaine saison de culture, à moins que l'altération du sol ne contribue de manière significative aux stocks de Ni.

Cycles biogéochimiques d'une parcelle tropicale d'agromine de Ni

Les plantes hyperaccumulatrices de Ni jouent un rôle majeur dans le recyclage du Ni dans les écosystèmes ultramafiques, qui peut être plus prononcé dans un environnement tropical humide. Les opérations d'agromine du nickel qui cultivent des plantes hyperaccumulatrices de nickel sur des substrats enrichis en nickel, y compris des sols ultramafiques, pour en extraire des produits de valeur, pourraient potentiellement affecter la dynamique du nickel dans ces

écosystèmes. Dans cette étude, nous étudions le cycle biogéochimique du Ni (et des nutriments) dans le cadre de scénarios d'agromine. *Phyllanthus rufuschaneyi* a été étudié dans la première ferme de métaux tropicale située à Bornéo (Sabah, Malaisie). Pendant deux ans, nous avons surveillé neuf parcelles de 25 m² et recueilli des informations sur les conditions météorologiques, l'exportation de biomasse, les flux d'eau et de litière vers le sol. Des manipulations de l'écosystème ont été réalisées pour tester la sensibilité de l'écosystème au retour de la litière dans le sol (doublement ou suppression). Sans récolte, les entrées et sorties de Ni ont des contributions mineures (< 1%) au budget total de Ni dans ce système. Le cycle du Ni est principalement déterminé par les flux internes, par la litière et les pluviollessivats. Après deux ans de récolte, le flux de Ni dans la litière correspondait à 50 % du stock total de Ni dans la biomasse aérienne (3,1 g m⁻² an⁻¹). Le Ni est lentement libéré de la litière ; après 15 mois de dégradation, 60 % de la biomasse initiale et 60-70% du Ni initial étaient encore présents. Le bilan du Ca, du P et du K est négatif ; sans fertilisation, une culture d'agromine durable conduirait à un épuisement du stock de Ca. L'élimination de la biomasse riche en P, K et Ca finira par affecter le rendement en Ni, et ces données doivent être prises en compte lors de la conception d'une culture d'hyperaccumulateurs tropicaux durable. L'altération pourrait reconstituer ce stock à long terme à partir des nutriments disponibles et doit être étudiée plus en détail.

Amélioration de l'agromine tropicale de Ni grâce à des fertilisants

L'agromine des "cultures métalliques" tropicales n'avait pas encore été testée, jusqu'à ce qu'un récent essai en pot soit entrepris pour tester la croissance et le rendement en nickel (Ni) de deux "cultures métalliques" prometteuses (*Phyllanthus rufuschaneyi* et *Rinorea cf. bengalensis*) à Sabah, en Malaisie. La présente étude examine la croissance et le rendement en nickel de *P. rufuschaneyi* en réponse à la fertilisation inorganique et organique dans des conditions de terrain. Une expérience de terrain de deux ans a été entreprise sur un Cambisol ultramafique à Sabah. Deux expériences ont été mises en place avec différents types d'engrais, organiques ou inorganiques. Dix parcelles de 9 m² ont été modifiées avec une fertilisation NPK (370 kg ha⁻¹) ou N (120 kg ha⁻¹) et ont fait l'objet d'un suivi pendant la première année. Une autre expérience avec des parcelles de 25 m² a été mise en place pour tester trois traitements d'amendement organique (0, 1 ou 2 kg m⁻² de biomasse de *P. rufuschaneyi* broyée). Les résultats montrent que malgré une variabilité considérable, la concentration en Ni était généralement plus faible dans les pousses de plantes amendées avec de l'urée uniquement. Cependant, les traitements n'ont montré aucun effet statistique sur la biomasse végétale et le rendement en Ni

à court terme, même si les traitements organiques ont eu un fort effet positif sur la disponibilité du Ni. Les effets à court terme des traitements inorganiques et organiques sur le rendement en Ni de la culture de *Phyllanthus rufuschaneyi* n'étaient pas apparents ($75 \text{ kg Ni ha}^{-1} \text{ an}^{-1}$). Les essais sur le terrain sont en cours pour évaluer les effets à long terme. Les résultats préliminaires suggèrent qu'avec des systèmes agronomiques et une sélection végétale appropriés, le rendement en Ni pourrait être considérablement augmenté.

C. DISCUSSION

a. Comportement des éléments

Le taux de litière dans cette forêt hyperaccumulatrice est comparable à la litière de nombreuses autres forêts tropicales humides ($1,1 \text{ kg m}^{-2} \text{ y}^{-1}$) (Bray JR et Gorham 1964, Burghouts et al. 1992, 1998). La majeure partie de la chute de litière a eu tendance à se produire entre mi-février et début avril 2019, ce qui correspond à la période la plus sèche de la région du Mont Kinabalu. Les quantités d'éléments contenues par la litière des feuilles de plantes hyperaccumulatrices ne différaient pas de celles des autres forêts tropicales (par exemple Proctor 1983), sauf pour le Ni. La litière est le principal flux d'apport de Ni dans les forêts naturelles à hyperaccumulation (77% ; $5,4 \text{ g m}^{-2} \text{ y}^{-1}$) et dans le système de culture ($3,1 \text{ g m}^{-2} \text{ y}^{-1}$), outre l'altération minérale qui n'a pas été étudiée ici.

Le Ni est légèrement plus concentré dans les vieilles feuilles que dans les jeunes feuilles et tend à être plus concentré dans la litière de feuilles que dans les feuilles sur pied. Cela pourrait être dû à une réabsorption limitée du Ni avant la sénescence, avec une accumulation de Ni dans les feuilles, due au phloème. La réabsorption des éléments était différente entre *P. rufuschaneyi* cultivé et sauvage, de sorte que les cycles biologiques étaient différents. Dans la forêt, les arbres ne réabsorbaient pas le Ca et le Mn comme ils le faisaient dans le système de culture. Cette réabsorption pourrait s'expliquer par les différentes interactions entre les éléments nutritifs dans les sols ou par l'impact de la récolte sur les différents stocks d'éléments. Cependant, le pluviollessivat est surestimé en raison de la couverture inconnue du sol de *P. rufuschaneyi*. Le budget annuel de K et Mn pourrait être négatif, ce qui explique le changement de leur cycle biologique. La réabsorption préférentielle de ces éléments pourrait être due à une diminution de leurs concentrations en éléments disponibles dans le sol en raison d'une récolte soutenue.

La concentration en nickel de la chute de litière des plantes traitées avec une forte ou une faible dose de litière est statistiquement égale à la concentration en Ni de la chute de litière

en milieu forestier de *P. rufuschaneyi*. Pour une plante non traitée avec de la litière, la chute de litière correspondait à 50 % de sa biomasse aérienne et était 50 % plus concentrée en Ni que pour les autres plantes des parcelles traitées avec de la litière. Pour les plantes traitées, la litière correspondait à 20-25% de leur biomasse aérienne, et pour la forêt endémique, la litière correspondait également à 20-25% de la biomasse aérienne. La récolte ne semble pas avoir d'impact sur le retour du Ni par la chute de litière, contrairement à la décomposition de la litière. Néanmoins, les conditions et l'âge des plantes ne sont pas les mêmes entre les deux sites.

Le taux de dégradation de la litière de feuilles mesuré dans la parcelle tropicale d'agromine est le même que le taux de dégradation de la litière de feuilles d'une forêt sur pied de *P. rufuschaneyi* ($k=1,1$). Après presque deux ans de culture, les micro-organismes peuvent dégrader la litière aussi rapidement que dans une forêt naturelle d'hyperaccumulateurs. En raison des petites mailles des sacs de litière utilisés, il a été démontré que l'effet du macroinvertébré sur *P. rufuschaneyi* était faible dans les deux sites. Le renouvellement du nickel était plutôt élevé dans la litière de feuilles dans les deux sites, même si la dégradation du bois est faible ($k=0,3$). Néanmoins, la dégradation de la litière des hyperaccumulateurs dans les écosystèmes tempérés semble être plus rapide (par exemple, Echevarria et al. 2018).

Dans la forêt naturelle des hyperaccumulateurs de Ni, aucune augmentation significative de la concentration en Ni total dans l'horizon supérieur n'était visible, malgré le taux élevé de litière (11 kg m^{-2}). Dans la première ferme de métaux tropicaux, après 15 mois de traitement avec de la litière, aucune différence significative dans la disponibilité du Ni entre le traitement à faible dose et le traitement à forte dose n'était visible. En tenant compte du fait que le traitement à faible dose a permis d'obtenir la même quantité de litière que dans une forêt d'hyperaccumulateur de Ni. La dégradation de la litière a augmenté la disponibilité du Ni dans les deux environnements, mais cette augmentation a été limitée. Sur le site de Pahu, nous avons pu suivre les eaux libres drainées à l'aide de lysimètres. Les sols amendés avec de la litière ont eu tendance à lixivier plus de Ni en dessous de 15 cm que les sols non amendés, bien que cela ne soit pas significatif ($p=0,11$). Cependant, à 30 cm, dans l'horizon B, la concentration de Ni était beaucoup plus faible dans les eaux de drainage qu'à 15 cm. La perte de nickel des horizons supérieurs est stockée dans l'horizon B. À long terme, l'horizon B pourrait gagner des stocks de Ni pour *P. rufuschaneyi*, bien que le Ni soit moins disponible que dans les horizons de surface à l'origine.

Après presque deux ans de culture, aucune diminution de la concentration en Ni DTPA n'a été constatée sur aucune des parcelles traitées, y compris le traitement sans retour. La disponibilité du Ni a même eu tendance à augmenter après la culture, avec un taux plus rapide pour la double dose que pour le traitement à dose unique. Cependant, ils semblent atteindre un plateau similaire après deux ans. La stabilité du stock de nickel disponible pourrait être due en partie au retour du Ni par la litière. Même dans le cadre de l'agromine, la litière ne peut être négligée, le retour de Ni correspond à 25-50% de la production de Ni par la récolte. En plus de la composition de l'horizon A, la composition de l'horizon B pourrait être un facteur clé pour l'exploitation agricole du nickel. La libération de Ni à partir de ce stock supérieur pourrait avoir un impact sur les opérations d'agro-industrie à long terme. Il doit également jouer un rôle actif dans l'alimentation des systèmes racinaires avec le Ni qui est retourné à la surface. L'autre retour qui pourrait influencer ce stock est l'altération. Nous n'avons pas mesuré ce flux dans cette étude, mais il est probablement crucial pour une compréhension complète de la boucle. Il devrait être ciblé dans les recherches futures. D'autres expériences pourraient être réalisées à différents endroits de la première ferme de métaux tropicaux, car la composition du substratum rocheux peut varier énormément (même sur un même site). Des indicateurs à la surface, autres que le Ni DTPA, pourraient être décrits pour donner une meilleure idée de l'altération du Ni. Il est essentiel d'établir des cultures de plantes hyperaccumulatrices sur des sols tropicaux ayant une bonne réserve de Ni disponible.

Améliorer l'agromine du Ni

Tous les sols ultramafiques ne peuvent pas supporter l'agro-industrie du nickel (Nkrumah 2018). L'amélioration de l'agromine peut se faire de nombreuses manières. Dans le cadre de ce projet, des fertilisations synthétiques et organiques ont été testées.

Au cours de cette étude, la récolte à différentes hauteurs a été testée ; un taillis à 50 cm semblait préférable pour une récolte durable. La récolte à un rythme plus fréquent qu'annuel n'est pas recommandée en raison d'une production de biomasse globalement plus faible. La récolte a permis d'augmenter le nombre de branches et la biomasse foliaire. Une récolte rapide est recommandée avant que les plantes n'atteignent 50 cm. En outre, *P. rufuschaneyi* est résistant aux pesticides courants, mais leur utilisation doit être évitée.

La fertilisation NPK peut améliorer la croissance des plantes hyperaccumulatrices dans les régions tempérées (Bani et al.2015a, b). Nos expériences ont démontrés aucun effet de l'addition de NPK sur le rendement en Ni pour une dose de 370 kg ha⁻¹. Ainsi, la fertilisation n'a pas modifié le comportement des plantes, à l'exception de l'ajout d'urée N. L'ajout d'urée à une dose de 120 kg ha⁻¹ a diminué la concentration de Ni dans les feuilles, comme cela a déjà été montré dans les pots par Nkrumah et al (2019b, c). Le Ni est nécessaire comme N pour l'activité uréase des plantes ; cette enzyme catalyse l'hydrolyse de l'urée. L'augmentation des concentrations de N pourrait peut-être diminuer le besoin des plantes hyperaccumulatrices en Ni en raison d'une activité uréasique plus faible. En outre, aucun effet sur le pH du sol des traitements n'a été détecté. Aucune fertilisation avec des nutriments n'est donc vraiment nécessaire pour améliorer le rendement en Ni de *Phyllanthus rufuschaneyi*, au moins après deux ans de culture. Cependant, elle pourrait s'avérer nécessaire pour équilibrer les flux sur le long terme.

Des traitements organiques et inorganiques ont été testés dans deux zones différentes de la première ferme de métaux tropicaux avec différentes teneurs en Ni DTPA dans la terre végétale. La fertilisation inorganique a été appliquée à des plantes poussant sur des sols dont la concentration en Ni disponible était inférieure (120 mg kg⁻¹) à celle des plantes fertilisées avec des matières organiques (160 mg kg⁻¹). Le rendement en Ni de ces deux parcelles était complètement différent, même pour le même traitement NPK (370 kg ha⁻¹). Le rendement en Ni de la parcelle contrôle du traitement litière était sept fois plus élevé (74 kg m⁻²) que le rendement en Ni du traitement NPK de l'amendement inorganique (10 kg m⁻²) pour la même densité (4 plantes m⁻²). Nous supposons que ce rendement plus faible en Ni est dû à une disponibilité plus faible du Ni. Une disponibilité de 160 mg kg⁻¹ de Ni serait le minimum pour avoir une agromine tropicale viable économiquement avec *P. rufuschaneyi*. Cette différence pourrait être due en partie à une différence de concentration totale de Ni, de composition de la phase contenant du Ni et de texture du sol. De plus, le sol du traitement inorganique avait tendance à avoir une texture plus argileuse. Des études supplémentaires sur les variations de la composition du sol pourraient aider à comprendre cet effet.

Perspectives de l'agromine tropicale

De nombreuses perspectives existent pour améliorer l'agromine en milieu tropical. Les pratiques agronomiques doivent être adaptées à chaque hyperaccumulateur cultivé. Le rendement en Ni obtenu dans cette étude (75 kg ha^{-1}) n'est qu'un résultat préliminaire, le rendement en Ni de l'Agromine tropical pourrait être durablement augmenté. La co-culture avec des légumineuses peut augmenter le rendement en Ni dans les régions tempérées (Saad et al. 2018) et pourrait également améliorer l'agromine en milieu tropical. Depuis le début de 2020, la co-culture entre *P. rufuschaneyi* et une légumineuse est testée sur le terrain de Pahu pour mesurer le potentiel de cette technique. Les légumineuses sont intéressantes pour leur capacité à augmenter la teneur en azote du sol, même si l'ajout chimique de cet élément diminue dans de nombreux cas la concentration de Ni dans les plantes. Des tests de densité sont également en cours dans la première ferme de métaux tropicaux. Il serait souhaitable de tester les pratiques agroforestières dans le cadre de futures expériences de culture. En outre, l'installation d'un système de drainage et d'irrigation afin d'améliorer la croissance de la biomasse pendant la saison sèche (février et mars) pourrait être mise en place pour tester l'effet sur l'augmentation du rendement en Ni.

Tous les flux de Ni n'ont pas été mesurés dans cette étude. Certains flux importants de Ni ont peut-être été négligés. Les flux d'altération pourraient être un flux critique pour comprendre le comportement du Ni dans l'environnement. Son calcul est nécessaire pour comprendre le comportement des hypernickelophores tropicaux dans les systèmes naturels et anthropiques. La modélisation des différents flux mesurés et du flux d'altération, ainsi que des études de texture plus approfondies, permettraient de confirmer la longévité de l'agromine tropicale et le budget des stocks disponibles.

The Ni biogeochemical cycle in a tropical agromining metal crop system

Hyperaccumulation is a phenomenon that was only discovered 45 years ago, it has been the focus of very intensive research because of the unusual behaviour of metals in the ecosystem and also because it offers a vast potential for nature-based solutions. Describing the Ni biogeochemical cycle within the soil-hyperaccumulator plants ecosystem is necessary to elucidate the ecological role of hyperaccumulator plants in their natural environment, but also to understand their potential behaviour under tropical agromining systems. Agromining and exporting Ni rich-biomass will interrupt the cycle. It is therefore important to understand the mechanisms which govern the Ni biogeochemical cycle in both natural and agromining systems: What are the Ni cycles (internal and external) and their impact on the ecological functioning of tropical hyperaccumulator forest? How rapid are the Ni fluxes across the soil-plant compartments, and what is the turnover of Ni in a hyperaccumulator tropical system? How fast can a tropical Agromining crop deplete Ni in soil? How can we manage soil fertilisation for a sustainable tropical Ni agromining crop? Therefore, the objectives were: (i) to study the biogeochemical cycling of a natural forest of *Phyllanthus rufuschaneyi* in order to assess and evaluate the natural fluxes of Ni in the ecosystem; (ii) to manipulate such an ecosystem in order to perform a sensitivity test of the ecosystem for the following flux: litter return to the soil; (iii) to optimize the cropping system of *P. rufuschaneyi* for Ni agromining. Two parallel stands of *P. rufuschaneyi* were instrumented, monitored and compared over two years (2018 and 2019), (i) a natural secondary 100-m² forest and (ii) a densely planted field in which litter returns to the soil were calibrated; from no return (export) to a doubling of the return. This study did not prove allelopathy of tropical hyperaccumulator plants, despite the extreme influence of Ni hyperaccumulators in building up available Ni stocks in topsoils. Nickel cycle was mainly driven by internal fluxes, *i.e.* degradation and recycling of the hyperaccumulator biomass. The percentage of Ni recycled by litterfall tended to decrease with increasing litter addition to the soil and was not influenced by coppicing, at least in the short term. Major nutrient (NPK) fertilisation did not affect Ni yield (*i.e.* 75kg Ni ha⁻¹ yr⁻¹) in the short term either, even if N fertilisation reduced Ni concentrations in leaves and plant biomass production. Nickel turnover should be taken into account when designing tropical agromining crops and natural secondary forests are a good surrogate to evaluate the long term impacts of agromining. Further study of the weathering processes would help to precise the contribution of bedrock and soil mineral horizons in the Ni and nutrient budgets of the system.

Keywords: biogeochemistry; Agromining; nickel hyperaccumulator plants, nutrient cycling, ultramafic soils

Le cycle biogéochimique du Ni dans un système d'agromine tropical

L'hyperaccumulation est un phénomène qui a été découvert il y a 45 ans seulement, il a fait l'objet de nombreuses recherches, en raison du comportement inhabituel des métaux dans l'écosystème et en raison du potentiel de solutions fondées sur la nature qui en découle. La description du cycle biogéochimique du Ni des plantes hyperaccumulatrices est nécessaire pour élucider leur rôle écologique dans leur environnement naturel, mais aussi pour comprendre leur comportement potentiel dans des cultures tropicales d'agromine. L'exploitation agricole et l'exportation de la biomasse riche en Ni interrompent le cycle du Ni. Il est donc important de comprendre les mécanismes qui régissent le cycle biogéochimique du Ni dans les systèmes naturels et de cultures : Quels sont les cycles du Ni et leur impact sur le fonctionnement écologique des forêts tropicales d'hyperaccumulateurs ? Quelle est la vitesse des flux de Ni à travers les compartiments sol- plante et quelle est la dynamique et le renouvellement du Ni dans un système tropical d'hyperaccumulateurs ? À quelle vitesse une culture tropicale Agromine peut-elle épuiser le Ni dans le sol ? Comment pouvons-nous gérer la fertilisation des sols pour une culture d'agromine tropicale de Ni durable ? Les objectifs de ce doctorat étaient donc les suivants (i) d'étudier le cycle biogéochimique d'une forêt naturelle de *Phyllanthus rufuschaneyi* afin d'évaluer les flux de Ni dans l'écosystème ; (ii) de manipuler un tel écosystème afin d'effectuer un test de sensibilité de l'écosystème pour le flux suivant : retour de la litière au sol ; de l'absence de retour (exportation) à un doublement du retour ; (iii) d'optimiser le système de culture de *P. rufuschaneyi* pour l'agromine du Ni. Deux peuplements parallèles de *P. rufuschaneyi* ont été instrumentés, surveillés et comparés pendant deux ans (2018 et 2019), (i) une forêt secondaire naturelle de 100 m² et (ii) un champ densément planté dans lequel les retours de litière au sol ont été calibrés ; de l'absence de retour (exportation) à un doublement du retour.

Cette étude n'a pas révélé l'allopathie des plantes hyperaccumulatrices tropicales. Les plantes hyperaccumulatrices de Ni influent sur la constitution des stocks de Ni disponibles dans les couches arables. Le cycle du Ni est principalement régi par des flux internes, notamment la dégradation et le recyclage de la litière. Ce pourcentage de renouvellement du Ni ne semble pas être influencé par le taillis à court terme mais par la présence initiale de litière sur le sol. La fertilisation NPK n'a aucun effet sur le rendement en Ni à court terme (75 kg Ni ha⁻¹ an⁻¹), même si la fertilisation en azote a tendance à réduire le rendement en Ni de *P. rufuschaneyi*. Il convient de tenir compte du renouvellement du nickel lors de la conception des cultures d'agromine tropicales. Une étude plus approfondie de l'altération permettrait de préciser son rôle dans la reconstitution des stocks de Ni disponible et de nutriments.

Mots-clefs : Biogéochimie , Agromine, Ni hyperaccumulation, cycles des nutriments, ultramafique