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Occurrence of mycorrhizal symbioses in the metal-rich lateritic soils of the Koniambo Massif, New Caledonia

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Abstract The occurrence of arbuscular mycorrhiza (AM) was surveyed in ten endemic plant species of the Koniambo Massif (New Caledonia) and associated metal-enriched ultramafic soils along a topographic sequence ranging from a plateau at 900 m altitude to a valley at 700 m. In the four different plant formations (*Araucaria* group on the plateau, ligno-herbaceous maquis, *Tristaniopsis* maquis and *Nothofagus* forest in the valley), all plants were consistently colonised by AM fungi, even the sedges *Costularia arundinacea*, *C. nervosa* and *Lepidosperma perteres* and the nickel-hyperaccumulating plant *Phyllanthus favieri*. Dual (AM and ectomycorrhiza EM) colonisation was observed in the two plant formations dominated by the ectomycorrhizal plants *Nothofagus balansae* for the forest (site 4) and *Tristaniopsis guillainii* and *T. calobuxus* for the *Tristaniopsis* maquis (site 3). In the soils, there are strong positive correlations between microbial activity, black AM spore abundance and concentrations of available metals indicating the role of the biotic component in the release of metals. These results suggest that these symbioses are important in the adaptation of the endemic plants to these soils, and may be relevant to ecological restoration of the ancient nickel mines.

Keywords New Caledonia · Arbuscular mycorrhiza · Ultramafic soils · Metals · Adaptation

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Introduction

A range of plants growing on heavy metal contaminated sites have been shown to form arbuscular mycorrhizas (AM) (Weissenhorn et al. 1995; Weiersbye et al. 1999; Khan et al. 2000; Turnau and Mesjasz-Przybylowicz 2003). This means that AM fungi have developed a tolerance towards metals, which are considered phytotoxic at these levels of concentrations. In lateritic soils, the study of AM has essentially focused on linking soil fertility with the presence of AM (Jasper et al. 1988, 1989; Brundrett et al. 1999). Very few studies link heavy metal concentrations to AM formation (Gonçalves et al. 2001; Turnau and Mesjasz-Przybylowicz 2003).

The New Caledonian tropical archipelago is an interesting case study, as it links thick metal rich lateritic soils (Fe, Ni, Cr, Co, Mn, Al) and an endemic flora developed on these oxisols (Jaffré 1980; Perrier et al. 2004). Initial studies on the soil microbiology of these endemic plants have shown the link between mobility of metals and total microbial activity (Amir and Pineau 2003a,b) and have tried to link mineralogy of the soils with the preferential liberation of metals by bacteria (Quantin et al. 2001, 2002). However, few ecosystems have been studied, and the mycorrhizal status of the endemic plants is yet to be defined to improve the processes of ecological restoration of ancient mine sites (Amir et al. 1997). Furthermore, the

presence of at least 40 species of nickel-hyperaccumulating plants (Jaffré 1980; Reeves 2003) shows the potential of this ecosystem in terms of phytomining. The discovery of AM colonisation in *Berkheya coddii*, a nickel-hyperaccumulating plant from South Africa, and the implication with respect to phytomining (Turnau and Mesjasz-Przybylowicz 2003; Krämer 2005), give an even greater importance to the study of the symbiotic status of the New Caledonian nickel hyperaccumulators.

In this specific environmental setting, the aim of this study is to highlight the AM status of ten dominant endemic plants of the Koniambo Massif (New Caledonia), to assess the quantity of AM spores in associated soils and to explore relationships between the activity of these soils, their heavy metal content and their AM status. The presence of key plant species for ecological restoration (located in every stratum of the plant formation) and a nickel hyperaccumulator will enable these results to be taken into account during the post-mining rehabilitation process.

Materials and methods

Study site

The Koniambo Massif, which is located in the Northern Province of New Caledonia (20°59′ S, 164°49′ E), is one of the isolated ultramafic massifs of the West coast (Fig. 1a,b). Here in the Koniambo Massif the mining consortium Falconbridge/SMSP established an exploration site for open-pit nickel mine and smeltery for high-grade nickel ore located in the thick regolith. The massif has a maximum altitude of 930 m and a total area of 381 km^2. The climate on the Koniambo Massif can be defined as tropical wet and dry (Aw), according to Köppen's climate classification

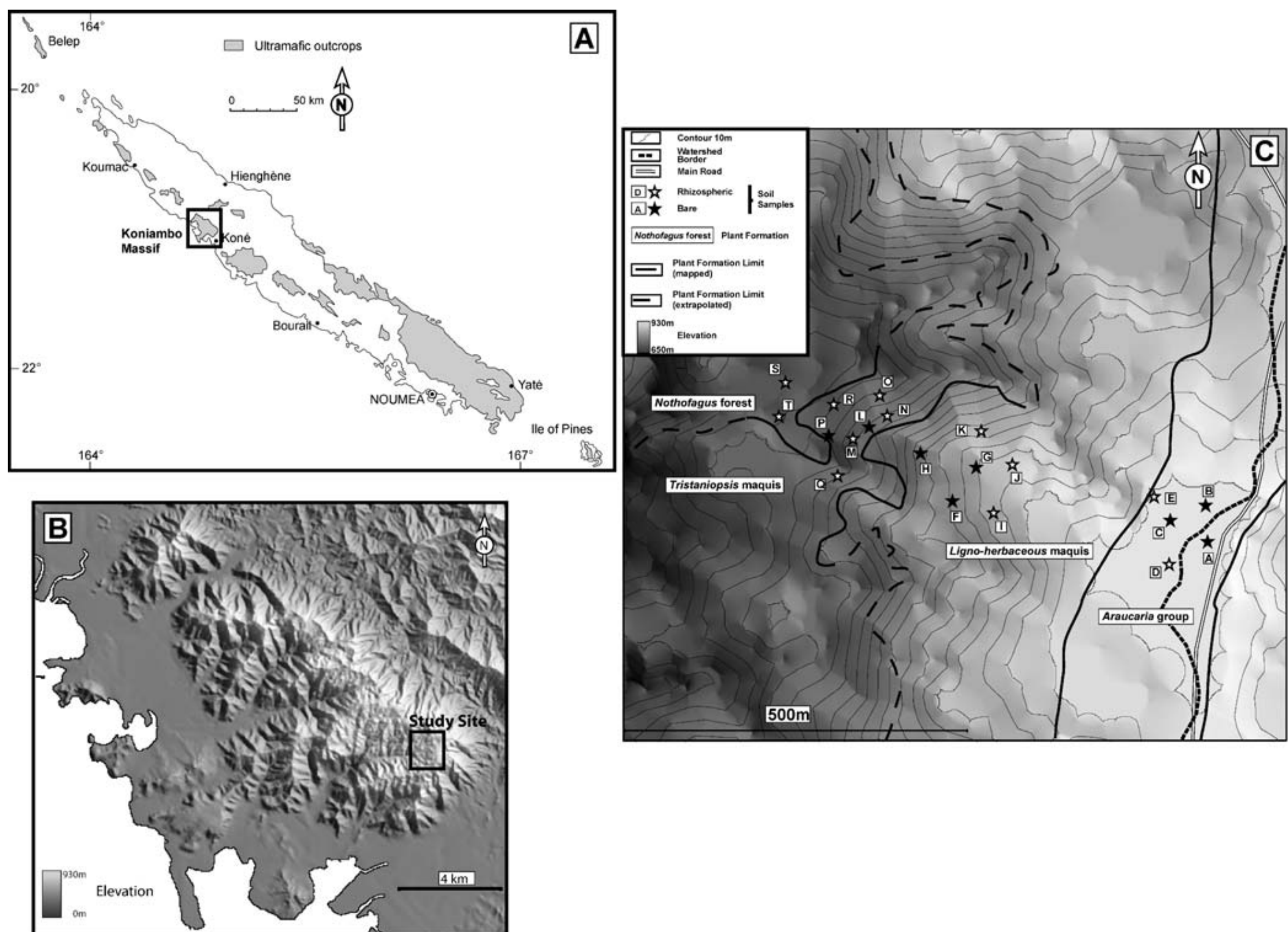

Fig. 1a–c: **a** Map of the ultramafic bodies of New Caledonia (in *grey*), **b** *shaded relief map* of the Koniambo Massif with study site location, **c** *shaded relief map* of the Trazy Guerioum zone of the Koniambo Massif with location of plant formations and sampling sites

(Köppen 1954) with two different seasons; a humid season ranging from January to April (an average of 250 mm monthly precipitation) and a drier season from May to December (an average of 100 mm monthly precipitation with a 50-mm low peak in September) (Meteo-France 2004). The wind regime is generally governed by the trade winds blowing from an ESE direction. The average daily temperature on the Koniambo Massif (at 900 m altitude) ranges from 22°C in the summer months (January to March) to 14°C in the winter months (July to September).

This ultramafic massif is mainly composed of a harzburgite substrate with some dunite and gabbro intrusions. Lateritic weathering of this body has led to the formation of a nickel-rich regolith varying in thickness according to the geomorphic setting (Roche 2001; Perrier et al. 2006). On the plateau areas, the weathering profile can be as thick as 70 m, which is comparable to the profiles studied by Trescases (1975) in the Kouaoua region. The soils present on the Koniambo Massif can be classified as highly weathered oxisols formed of a majority of iron oxides (goethites in majority). These soils are characterised by a deficiency in the main plant nutrients; *P* values have a maximum of 30 $\mu g\ g^{-1}$, K of 40 $\mu g\ g^{-1}$, Na of 20 $\mu g\ g^{-1}$ and Ca of 100 $\mu g\ g^{-1}$; and high concentrations of metals mainly Fe of 600 $mg\ g^{-1}$, Al of 30 $mg\ g^{-1}$, Cr of 20 $mg\ g^{-1}$, Ni of 10 $mg\ g^{-1}$ and Co of 2 $mg\ g^{-1}$ (Perrier et al. 2006). The remaining characteristics are shown in Table 3.

Six hundred and fifty plant species were numbered on the Koniambo Massif (Roche 2001) and 12 different vegetation formations were described by Jaffré (1974) according to plant abundance. The vegetation is an assemblage of maquis and rain forest; 90% of the plant species are endemic to New Caledonia.

We chose to study a topographic sequence that ranges from the summit plateau at 900 m to a valley at 700 m (Fig. 1c). The vegetation on this watershed is native and has been affected by the mining activity only for prospection reasons. It is formed of four different vegetation groups defined by Jaffré (1974): *Araucaria montana* group, ligno-herbaceous maquis, *Tristaniopsis* maquis and a *Nothofagus* forest. Within these groups a number of species were chosen according to various parameters, abundance in the different plant formations and in the vegetation strata and potential for use in ecological restoration (Jaffré 1992). These perennial plant species, which are all endemic to New Caledonia, are (Fig. 2): *Araucaria montana* Brongn. (Araucariaceae) dominant (in terms of cover abundance) in site 1; *Carpolepis laurifolia* J. W. Dawson (Myrtaceae); *Costularia arundinacea* Kuek. (Cyperaceae), a ubiquitous sedge on ultramafics; *Costularia nervosa* Raynal (Cyperaceae), dominant in site 2; *Lepidosperma perteres* C.B. Clarke (Cyperaceae); *Codia montana* Forster and G. Forster (Cunoniaceae), dominant in site 2; *Tristaniopsis guillainii* Viell. ex Brongn. and Gris (Myrtaceae), dominant in site 3;

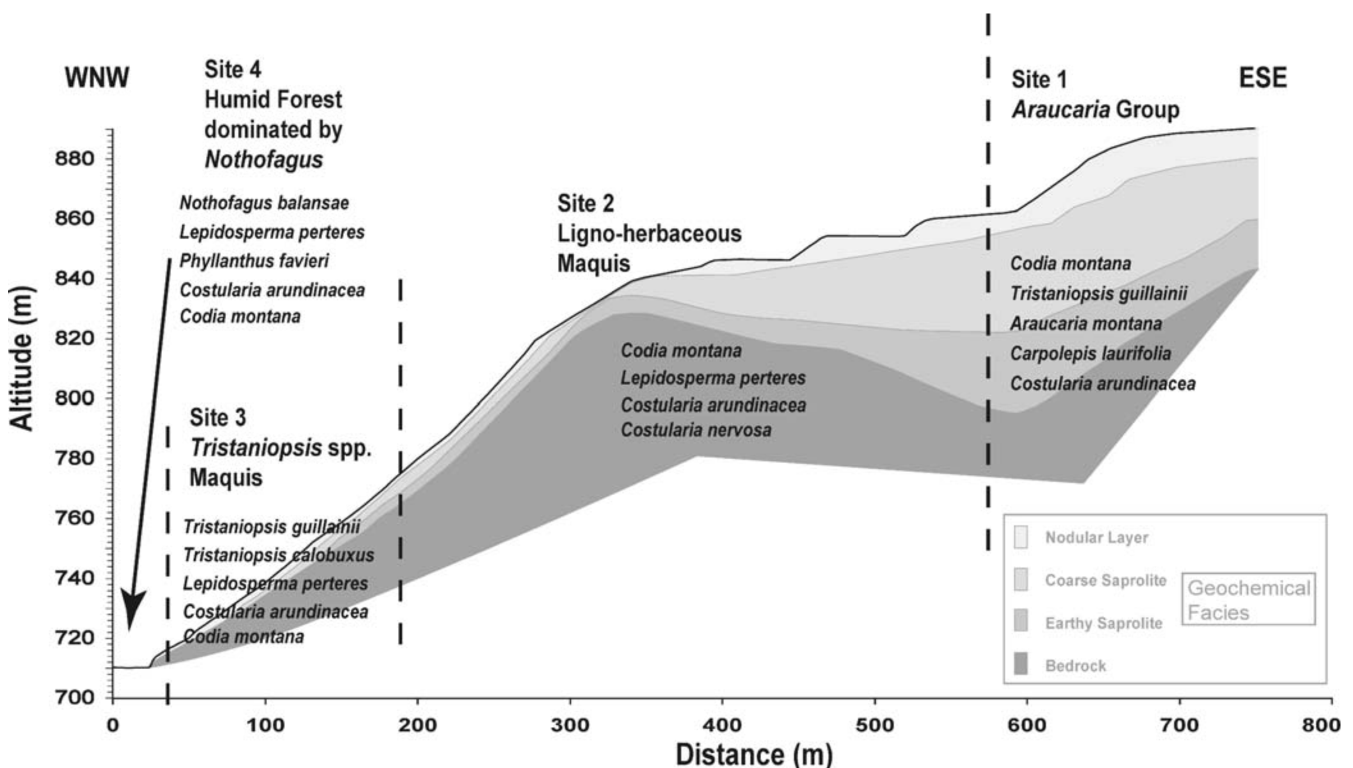

Fig. 2 Distribution of the plant species within the four vegetation groups along the studied topographic sequence overlying the geochemical cross-section of the regoliths; distance (*abscissa*) is represented from the valley to the summit plateau

Tristaniopsis calobuxus Brongn. and Gris (Myrtaceae), dominant in site 3; *Phyllanthus favieri* M. Schmid (Euphorbiaceae, known nickel hyperaccumulator located in site 4) and *Nothofagus balansae* Steenis (Fagaceae), dominant in site 4.

Roots

The sampling was conducted in April 2003 and 2004, according to the different vegetation groups established previously (Figs. 1c, and 2). Four sites were sampled along the topographic sequence corresponding to the four different vegetation and soil facies. Eight randomly chosen shoots of each plant species were selected per site. The surface explored for each site and each species was about 1 ha and the distance between the shoots was 10 to 30 m. The fine roots were sampled and placed in fresh plastic bags. To avoid confusion between the roots of different plants, the plants were dug up and the roots were followed from the main root to the finer roots, which were sampled, then replanted. The roots were kept refrigerated until treatment. In the laboratory, roots were prepared following the procedure of Koske and Gemma (1989). After being thoroughly rinsed with water, roots were immersed in a 10% KOH bath at 90°C for 90 min, a few drops of H_2O_2 were added after 1 h to lighten the colour; they were then rinsed again and stained by immersion in a trypan blue bath for 15 min at 90°C. For each sample, 30 stained segments (approximately 1 cm long) randomly selected were observed under the microscope. A root was considered AM when arbuscules, vesicles or hyphal coils were present. However, the oxidic nature of the soils in which the roots were lying (dominance of iron oxy-hydroxides dark red in colour) made the visualisation of arbuscules difficult. AM infection percentage (*F*) and AM intensity percentage (*M*) were determined according to the method of Trouvelot et al. (1986). These two parameters are:

$$F(\%) = \frac{number\ of\ mycorrhizal\ root\ fragments}{total\ number\ of\ root\ fragments} \Big/ 100$$

$$M(\%) = \frac{95n5 + 70n4 + 30n3 + 5n2 + n1}{total\ number\ of\ root\ fragments}$$

where $n5$ is the number of root fragments with more than 90% infection, $n4$ between 90 and 50% infection, $n3$ between 50 and 10% infection, $n2$ between 10 and 1% infection, $n1$ less than 1% infection.

Soils

Soils were sampled in each of the four sites (amounting to 20 samples in total). The top 20 cm was taken from both rhizospheric (high root density) and bare soils. After a thorough mix, all samples were sieved through a 2-mm mesh before treatment. To assess the availability of metals, two chemical extractions were performed following the Lindsay and Norvell (1978) method modified by Bourdon et al. (1995): KCl 1 M solution in a 1/10 soil solution (v/m) ratio (for the exchangeable metals) and diethylene triamine pentacetic acid (DTPA) 0.005 M + $CaCl_2$ 0.01 M solution in a 1/5 soil solution (v/m) ratio (for metals adsorbed or complexed on the soil organic or mineral phases), followed by measurement with an ICP-OES (inductively coupled plasma-optical emission spectroscopy) for these elements: Ni, Fe, Al, Cr, Mn, Ti, Co, Mg and Cu. Soil pH was measured both in water and buffered with KCl (1/2.5 soil solution v/m ratio). Total C and N were measured using an automatic CHNOS analyser (Thermoquest Finnigan). All chemical analyses were performed at the chemistry laboratory of IRD Noumea.

The FDA (fluorescein diacetate activity) enzymatic method is frequently used for comparative studies of total microbial activity in soils (Schnürer and Rosswall 1982; Zelles et al. 1987). The FDA test was performed as follows: a well-mixed soil sample (1 g) was added to a 10-ml phosphate buffer at pH 7.6 (KH_2PO_4 0.1 N; Na_2HPO_4 0.1 N) and homogenised by vortex for 1 min before incubation for 20 min at 23°C. To the soil suspension 1 ml FDA (fluorescein diacetate 200 mg, acetone 60 ml, distilled water up to 100 ml) was added. The tubes were incubated again for 40 min at 23°C in the dark. The reaction was stopped by centrifugation at 2,500×*g* for 10 min and filtered through Whatman paper GF/F. The absorbance was measured with a spectrophotometer at 490 nm. The amount of fluorescein in the filtrate was determined from a standard curve (490 nm) and FDA hydrolysis activity was expressed as nanomol (nmol) of fluorescein h^{-1} g^{-1} of soil (dry weight). Three replicates were prepared for each sample.

The numbers of AM spores present in each soil sample were determined after wet-sieving and centrifugation (Tommerup and Kidby 1979). The minimum sieve mesh was 38 mm. Only spores filled with intact cytoplasm were recorded. The AM spores were separated into two groups according to their colour, light to brown spores (Lsp group) on one hand and black spores (Bsp group) on the other. These two groups could have an ecological significance, as the light to brown spores generally still possess a germination potential, whereas the black spores have lost the ability to germinate (Amir, unpublished data).

There was special focus on the nickel hyperaccumulator *Phyllanthus favieri* located in site 4. Nine shoots were randomly chosen and fine roots were collected. The leaf concentrations of nickel were analysed by ICP-OES after an acid dissolution (aqua regia attack in a microwave oven); the rhizospheric soils under *Phyllanthus* were sampled to a

depth of 15 cm, the number of AM spores and the Ni concentrations extracted by DTPA were determined.

Results

Mycorrhizal status

Arbuscular mycorrhizal colonisation was evident in all plant roots collected. However, arbuscules were rarely observed due to the relatively dark colour of the roots even after the clearing effect of the H_2O_2 treatment. Vesicles and aseptate intracellular hyphae were present in the roots of all studied species. Appressoria were also observed. Besides AM, *Tristaniopsis calobuxus*, *T. guillainii* and *Nothofagus balansae* were ectomycorrhizal. Roots of *Costularia arundinacea* also had ectomycorrhizas on short secondary roots (septate hyphae and fungal sheath).

Table 1 shows the data for the ecology and mycorrhizal status of the different plant species. The highest values of AM frequency (*F*) were noted for sites 1 and 2. *Araucaria Montana* had the highest value (92.6%) followed by *Carpolepis laurifolia* (69.8%), *Costularia arundinacea* (58.1%), *Codia montana* (51.5%) and *Tristaniopsis guillainii* (35.1%). *Costularia arundinacea* and *Codia montana* were present and colonised in all vegetation formations and they had higher *F* values in sites 1 and 2: 5respectively, 8.1 and 53.1% for *Costularia* and 51.5 and 55.8% for *Codia*. *Costularia nervosa*, the most abundant species in site 2, had also the greatest values of *F* (57.1%) in this site. *Lepidosperma perteres* present in sites 2, 3 and 4 had the greatest *F* values (41.3%) in site 3. *Tristaniopsis guillainii* is also located in site 3, its *F* value much lower (24.7%) than for site 1, but dual colonisation (AM and EM) was observed in this site. Dual colonisation was also observed for *Tristaniopsis calobuxus* and *Costularia arundinacea* in this site; values for *F* are similar (16.5 and 22.7%, respectively). The dominant species in site 4 is *Nothofagus balansae*; it had the smallest value for mycorrhizal frequency (7.5%) and had dual colonisation. The values of intensity of root colonisation M were relatively low (below 20%) except for *Araucaria montana* (33.1%); however, these values followed closely the variations of *F*. Hence, lower values were observed for sites 3 and 4.

Data for root colonisation of the nickel hyperaccumulator *Phyllanthus favieri* are shown in Table 2. Variations of colonisation can be observed, with some values of *F* greater than 50%, whereas other samples show no colonisation. The *M* values were much lower but varied in a similar way to the *F* values. The Ni leaf concentrations were very high,

Table 1 Studied plant species in their ecological setting with the mycorrhizal patterns of colonisation

Family	Species	Site	Ecological type	Plant type/ canopy strata	VAM colonisation		EM colonisation
					F%	*M*%	
Araucariaceae	*Araucaria montana* Brongn.	1	Maquis	A/Upper	92.6	33.1	−
Cyperaceae	*Costularia arundinacea* Kuek.	1	Maquis	H/Ground	58.1	6.3	−
Cunoniaceae	*Codia montana* Forster & G. Forster	1	Maquis	S/Ground	51.5	0.8	−
Myrtaceae	*Carpolepis laurifolia* J.W. Dawson	1	Maquis	T/Middle	69.8	19.1	−
Myrtaceae	*Tristaniopsis guillainii* Viell. ex Brongn. & Gris	1	Maquis	S/Ground	35.1	5.2	−
Cyperaceae	*Costularia arundinacea* Kuek.	2	Maquis	H/Ground	53.4	9.6	−
Cyperaceae	*Costularia nervosa* Raynal	2	Maquis	H/Ground	57.1	11.8	−
Cyperaceae	*Lepidosperma perteres* C.B. Clarke	2	Maquis	H/Ground	16.7	0.4	−
Cunoniaceae	*Codia montana* Forster & G. Forster	2	Maquis	S/Ground	55.8	15.9	−
Cyperaceae	*Costularia arundinacea* Kuek.	3	Maquis	H/Ground	22.7	0.4	+
Cyperaceae	*Lepidosperma perteres* C.B. Clarke	3	Maquis	H/Ground	41.3	10.6	−
Cunoniaceae	*Codia montana* Forster & G. Forster	3	Maquis	S/Ground	48.6	8.9	−
Myrtaceae	*Tristaniopsis calobuxus* Brongn. & Gris	3	Maquis	T/Middle	16.5	3.1	+ (abundant(1))
Myrtaceae	*Tristaniopsis guillainii* Viell. ex Brongn. & Gris	3	Maquis	T/Middle	24.7	3.1	+ (abundant(1))
Cyperaceae	*Costularia arundinacea* Kuek.	4	Forest	H/Ground	38.5	10.7	+
Cyperaceae	*Lepidosperma perteres* C.B. Clarke	4	Forest	H/Ground	14.7	0.3	−
Cunoniaceae	*Codia montana* Forster & G. Forster	4	Forest	S/Ground	40.7	1.3	−
Nothofagaceae	*Nothofagus balansae* Steenis	4	Forest	A/Upper	7.5	0.7	+(abundant(1))

(1) Confirmed by Perrier et al. (unpublished results)

Plant type: *A* Arboreal, *T* short tree, *S* Shrub, *H* Herbaceous; AM colonisation: *F*% mycorrhizal frequency, *M*% mycorrhizal intensity; EM colonisation: − absence of EM, + presence of EM

Table 2 Variation of the mycorrhizal colonisation of the Ni hyperraccumulator *Phyllanthus favieri* (located in site 4) in relation with Ni concentration in the leaves, Ni extracted by DTPA from the soils and AM spores in soils

Plant sample	*F* %	M %	Ni (in leaves) μg g^{-1}	Spores Number of spores/ 100 g soil	DTPANi (soil) μg g^{-1}
1	62.1	9.7	13,715	4,333	129
2	0	0	22,032	638	138
3	9.2	0.1	10,537	3,627	96
4	49.3	16.5	14,297	4,600	100
5	0	0	36,046	408	199
6	0	0	42,213	287	223
7	50.6	10	10,003	2,885	91
8	48.4	5.8	19,516	nd	nd
9	30.3	0.7	27,407	734	86

F Mycorrhizal frequency, *M* mycorrhizal intensity, *nd* not determined

ranging from 10,003 to 42,213 μg g^{-1}. The three samples, 2, 5 and 6, devoid of AM colonisation, showed leaf Ni content greater than 20,000 μg g^{-1}. The samples showing the greatest values of AM colonisation (1, 4 and 7) had the lowest concentrations in the leaves (<15,000 μg g^{-1}). The concentrations of DTPA extractable Ni in soils were relatively high, especially in the soils under the non-colonised plants.

Biogeochemistry of the soils

Results of the organic characteristics and geochemical characteristics of the soils are shown in Table 3. The numbers of AM light to brown spores (LSp) per 100 g of soil ranged from 0 to 665 in the bare soils, whereas in the rhizospheric soils they ranged from 1,077 to 4,687 (Table 3). The distribution was homogeneous between the different sampling sites both for bare and rhizospheric soils. For the AM black spores (BSp), in the bare soils they ranged from 63 to 1,393, whereas in the rhizospheric soils they ranged from 2,153 to 7,315. Similar to the light to brown spores (LSp), their distribution was homogeneous throughout the sequence, except for the *Tristaniopsis* maquis, which had the greatest spore counts for the bare soils (1,393 and 823). Three different spore genera were noted based on morphologic characteristic, *Glomus*, *Acaulospora* and *Gigaspora*. The most common genus was *Glomus*; more than 90% of the spores collected from these soils belonged to this genus. The total microbial activity (FDA) values followed the same patterns as the spore counts. In the bare soils, they ranged from 0.4 to 184.8 nmol of fluorescein h^{-1} g^{-1} of soil, but in the rhizospheric soil they ranged from 65.3 to 769.5 nmol of fluorescein h^{-1} g^{-1} of soil.

The concentrations of carbon and nitrogen had a similar distribution; they were greatest for the forest soils (10.55 and 20.54% for total organic C and 0.43 and 0.4% for N) and lowest for the bare soils. In the bare soils, the pH values ranged from 4.3 to 6.8; they were the lowest in site 1. In the rhizospheric soils, they ranged from 4.6 to 6.2, and the distribution was homogeneous between the different sites.

Two different forms of metal were analysed: the exchangeable fraction by KCl for Mn and Ni, and DTPA for metals adsorbed on the soil organic or mineral phases (for Mn, Co, Ni, Mg, and Fe) (Table 3). In the bare soils, whatever the position along the sequence, the exchangeable Ni concentrations were nil. In the rhizospheric soils, apart from site 1 for which the concentrations were nil, the amounts ranged from 2 to 17 μg g^{-1}. A similar distribution was observed for Mn extracts in the bare soils except for sample P in site 3. For the rhizospheric soils, the greatest concentrations were recorded in the forest soils (124 and 186 μg g^{-1}).

For the DTPA extracts, the amounts of metals were low for site 1 except for Fe with amounts ranging from 22 to 90 μg g^{-1}, Mg had greater values in the rhizospheric soils than in the bare soils, but they remained relatively low (13 and 20 μg g^{-1}). For the rest of the sequence, the amounts of all extracted metals were lower in the bare soils than in the rhizospheric soils. The forest soils (samples S, T) released the most metals of whatever type.

The total correlations between the different parameters are expressed in Table 4. Underlined are the statistically significant coefficients (p=0.05). Of interest here are the relationships between the extractable metals and the organic parameters. There were high correlation coefficients between N and all the extracted metals; it was similar for C except for Co (extracted with DTPA). There was no significant correlation between the soil AM spore numbers and organic N and C; however, there was a significant positive correlation with the FDA (0.472 and 0.445, respectively, for LSp and BSp).

The LSp values were positively correlated to Co (extracted with DTPA), but the coefficients with all the other metals were insignificant. In contrast, the BSp values were positively correlated to Ni, Mn, Mg, Co (extracted with DTPA) and Ni (extracted with KCl). The FDA was

Table 3 Different biological and geochemical parameters of the studied soils

Sample	LSp Number of spores/ 100 g soil	BSp Number of spores/ 100 g soil	FDA nmol fluorescein/ h/g soil	H_2O pH	N total %	C total %	DTPAMn µg g^{-1}	DTPANi µg g^{-1}	DTPAMg µg g^{-1}	DTPAFe µg g^{-1}	DTPACo µg g^{-1}	KCLMn µg g^{-1}	KClNi µg g^{-1}
A (1B)	32 (29)	63 (29)	13.3 (4.2)	4.4	0.03	1.54	0	0	1	22	0	0	0
B (1B)	570 (176)	507 (177)	5.5 (2.3)	4.3	0.01	0.61	0	0	2	43	0	0	0
C (1B)	253 (57)	633 (259)	7.7 (7.6)	4.3	0.01	0.52	0	0	2	48	0	0	0
D (1R)	2565 (197)	5763 (413)	154.9 (19.5)	4.6	0.01	1.85	4	1	13	90	0	5	0
E (1R)	2882 (562)	3942 (361)	120.5 (27.1)	5.0	0.01	1.37	11	1	20	44	1	7	0
F (2B)	538 (79)	253 (58)	184.8 (28.6)	5.6	0.03	1.60	39	3	21	47	10	1	0
G (2B)	63 (33)	285 (0)	70.5 (58.9)	5.0	0.02	0.90	14	1	12	25	2	1	0
H (2B)	0 (0)	63 (55)	21.0 (2.2)	4.9	0.00	0.40	14	0	0	42	2	1	0
I (2R)	1362 (58)	4528 (551)	463.2 (16.4)	5.7	0.24	5.85	512	118	242	90	61	24	12
J (2R)	4687 (331)	5858 (419)	286.4 (8.1)	6.2	0.14	3.68	372	127	504	80	117	7	4
K (2R)	2977 (1653)	3309 (1165)	169.5 (78.1)	4.9	0.06	2.49	173	14	98	74	24	14	2
L (3B)	665 (219)	1393 (464)	0.4 (3.0)	6.8	0.00	0.21	37	12	221	30	11	0	0
M (3R)	4338 (227)	4053 (388)	247.7 (49.9)	5.3	0.10	3.77	384	56	208	112	54	38	11
N (3R)	3293 (257)	5146 (237)	446.6 (2.2)	5.6	0.11	3.78	317	46	108	103	47	24	12
O (3R)	2723 (703)	7315 (848)	338.1 (40.0)	4.7	0.03	1.67	625	11	21	124	49	33	2
P (3B)	285 (326)	823 (1073)	44.5 (14.4)	4.8	0.03	2.70	23	1	6	25	1	12	0
Q (3R)	2027 (57)	3673 (44)	65.3 (16.5)	5.5	0.03	1.56	324	3	19	83	16	14	0
R (3R)	2217 (132)	4623 (269)	117.9 (21.6)	5.2	0.01	1.51	113	1	16	47	4	36	0
S (4R)	1077 (496)	6903 (5583)	221.9 (24.4)	5.7	0.43	10.55	1213	246	818	143	120	124	17
T (4R)	2122 (88)	2153 (603)	769.5 (123.9)	5.1	0.40	20.54	231	34	165	558	8	186	15

Values in brackets are standard deviations

Samples: (1) site 1, (2) site 2, (3) site 3, (4) site 4

B Bare soil, *R* rhizospheric soil (see Fig. 1c), *LSP* light to brown spores, *BSp* black spores, *FDA* fluorescein diacetate activity

Table 4 Correlation matrix between the different biological and geochemical parameters of the studied soils, for 20 observations (see Table 3)

	LSp	BSp	FDA	pH	N total	C total	DTPANi	DTPAMn	DTPAMg	DTPAFe	DTPACo	KClMn
BSp	0.727	1										
FDA	0.472	0.445	1									
pH	0.269	0.273	0.22	1								
N total	0.163	0.375	0.722	0.287	1							
C total	0.171	0.218	0.809	0.139	0.904	1						
DTPANi	0.301	0.715	0.371	0.463	0.791	0.473	1					
DTPAMn	0.238	0.556	0.43	0.34	0.722	0.448	0.865	1				
DTPAMg	0.267	0.51	0.301	0.572	0.75	0.467	0.958	0.801	1			
DTPAFe	0.24	0.176	0.821	0.034	0.72	0.93	0.2	0.269	0.22	1		
DTPACo	0.494	0.683	0.39	0.519	0.613	0.31	0.906	0.849	0.886	0.118	1	
KClMn	0.154	0.294	0.72	0.106	0.874	0.954	0.477	0.543	0.485	0.895	0.31	1
KClNi	0.328	0.455	0.77	0.317	0.905	0.789	0.752	0.728	0.678	0.632	0.64	0.766

Underlined coefficients significant at p=0.05; for significance at p=0.01, r=0.5368; at p=0.001, r=0.6524

positively correlated to Mn and Fe (extracted with DTPA) and to Mn and Ni (extracted with KCl).

Discussion

This is the first report of the occurrence of mycorrhiza in the ten endemic metallophytes studied in this paper. Whatever their position within the plant formations, all plant species were found to form AM symbioses. The occurrence of AM in the sedges *Costularia arundinacea*, *C. nervosa* and *Lepidosperma perteres* further invalidates the conclusions of Powell (1974, 1975) that sedges are fundamentally non-mycorrhizal. Muthukumar et al. (2004) have shown that the relationship between the sedge group and mycorrhizal symbionts (both EM and AM) has to be reconsidered, in that some species seem never to be colonised by mycorrhizal fungi, and others have different levels of affinity.

Furthermore, according to the same authors, environmental conditions seem to be the principal factor influencing the mycorrhizal status of these plants. In the extreme soil conditions in which the sedges were found, it is possible that the fungus is the best means for the plant to access the very scarce pools of P (Bolan et al. 1987) and N (Ames et al. 1983; Marschner and Dell 1994). Although no hartig net was observed, an EM fungal mantle on the first-order lateral roots of *Costularia arundinacea* was shown. This association was only observed in sites 3 and 4, which were dominated by the ectomycorrhizal plant species *Tristaniopsis guillanii* and *Nothofagus balansae*, respectively. Thus, it is possible that the affinity between *Costularia arundinacea* and EM fungi is low, infection being a result of the proximity with the abundant fungal associations of the neighbour species. EM associations have already been shown for the Cyperaceae genus *Carex* (Harrington and Mitchell 2002). However, future works need to focus on these associations in New Caledonia to confirm the actual EM symbiosis in the genus *Costularia*.

Another interesting association is the presence of AM in the roots of the nickel-hyperaccumulating plant *Phyllanthus favieri* (Euphorbiaceae), which can accumulate up to 40,000 μg g^{-1} Ni dry weight in its leaves (Table 2). To our knowledge, this is the second study showing mycorrhizal symbiosis between a Ni-hyperaccumulating plant and AM, the first being the Ni hyperaccumulators from South Africa (Turnau and Mesjasz-Przybylowicz 2003). These authors studied plants from the Asteraceae family, especially *Berkheya coddii*, and showed an increase in shoot biomass and shoot Ni content for plants colonised by AM fungi under laboratory conditions. However, in comparison with the samples of *Phyllanthus favieri* collected in the field, these plants contain lower concentrations of Ni in the shoots with a maximum of 13,200 μg g^{-1}. In *Phyllanthus favieri*, the plants were not mycorrhizal above 30,000 μg g^{-1} Ni in the leaves. This could mean that there is a hyperaccumulation threshold above in which AM are not formed for this endemic New Caledonian plant.

This hypothesis is further suggested by observations on seven other Ni-hyperaccumulating plants from New Caledonia (Collion 2004). The inhibition of mycorrhization could be a result of increased bioavailable Ni in the litter below the *Phyllanthus favieri* plants containing the most Ni, inducing a strong selection on the microflora as Schlegel et al. (1991) have shown for bacteria in similar soils from New Caledonia. Our results indicate that high concentrations of available Ni probably induce a reduction in AM fungi spores, as the highest concentrations of DTPA Ni (223 μg g^{-1}) was measured in the sample containing the lower number of AM spores (Table 2). More research is needed to understand the relationships between the AM colonisation and the hyperaccumulation of Ni by

some of these endemic plants. Indeed, as 75% of the vegetation of New Caledonia is endemic, these fungi have most probably evolved in parallel and have developed specific mechanisms to control the high metal and low nutrient concentrations.

Overall, there was no clear link between the mycorrhizal status of the different plant species (Table 1) and the level of concentrations of metals in the soils of each site (Table 3). The mycorrhizal infectivity should instead be related to the affinities of the different plant species to AM and EM fungi. Therefore, it is highly possible that the competition between AM and EM mycelia is preponderant in the colonisation of the plant roots with AM fungi. Indeed, our results show that ectomycorrhizal plant species have much smaller values of AM colonisation, especially for sites 3 and 4 (*F* and *M*, Table 1). In these sites, dominated by ectomycorrhizal plants, the abundance of ectomycorrhizal mycelium has probably lowered the infection potential of AM fungi. The absence of ectomycorrhizal symbiosis in sites 1 and 2 could be a result of greater exposure to the sun lowering the ability of EM fungi to bear sporophores. Indeed, site 1 is a rocky plateau with a loose maquis and site 2 is a maquis dominated by sedges where dry conditions prevail. *Tristaniopsis guillainii*, a known ectomycorrhizal plant species in sites 3 and 4 does not show any infection by EM fungi in site 1. In contrast, sites 3 and 4, which are dominated by ectomycorrhizal plant species and in which sporophores were reported (Perrier et al., unpublished results), are more humid with taller vegetation up to 3 m in the maquis in site 3 and up to 20 m in the forest in site 4. These conditions favour the development of ectomycorrhiza as was suggested by Erland and Taylor (2002).

The total correlations presented in Table 4 show that the abundance of AM spores and the total microbial activity (FDA) are correlated to a number of abiotic factors. The correlation between the FDA and the concentration in carbon and nitrogen shows that the rhizospheric soils are more microbially active than the bare soils. The positive correlation of the FDA with metal concentrations (Mn, Fe, Ni) shows that microbial activity increases the release of metals (Amir and Pineau 2003b); it can also explain the increase in available metals within the rhizospheric soils compared to the bare soils. This is in agreement with the results of Perrier et al. (2006), which show that mobility of metals in these soils is related to organic matter. Moreover, there is a positive correlation between the concentrations of available metals (mainly Ni and Co) and Black spore abundance. This result confirms the hypothesis of Amir et al. (1997) according to which the black colour of the AM spores is linked to the adaptation of these spores to the high concentrations of metals in these soils. Melanisation of the spores has already been shown by Daniels-Hetrick (1984) and Brundrett (1991) to be a reaction to various stress factors.

In summary, this study has shown that AM fungi formed mycorrhizal symbioses with ten plant species endemic to New Caledonia growing on highly metalliferous nutrient-depleted ultramafic soils. Three species belong to the Cyperaceae family, previously thought to have very low affinity with fungal symbionts, and one is a nickel hyperaccumulator. There was a high density of AM spores in the soils although concentrations of available metals are high, indicating a possible adaptation of these symbionts to the soils. However, further studies need to continue the characterisation of these symbionts to understand the adaptation mechanisms to these extreme soils. Furthermore, the use of these presumably adapted symbionts through inoculation in the ecological restoration of mine sites after exploitation seems valuable to guarantee a greater success of this process.

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