A comparative study of flora and symbiotic microflora diversity in two *Gymnostoma* formations on ultramafic rocks in New Caledonia

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In this study we compared the diversity of the flora and microsymbiotic flora (*Frankia*) found in vegetation formations dominated by two Casuarinaceae species: *Gymnostoma chamaecyparis* (Poisson) L. Johnson and *Gymnostoma deplancheanum* (Miquel) L. Johnson. *G. chamaecyparis* and *G. deplancheanum* are both endemic to ultramafic soils in New Caledonia. The first occurs only on hypermagnesian soil, and the second on iron crust oxisol. Vegetation formations containing these species are floristically very different. The flora found in *G. chamaecyparis* formations is more diverse and specialized than the flora associated with *G. deplancheanum.* The genetic patterns found in nodules of these two species of *Gymnostoma* were largely ubiquitous. The diversity observed for the flora, in relation to edaphic conditions, was not found for the *Frankia* strains associated with the two species of *Gymnostoma.* The difference in *Frankia* genetic diversity between the *Gymnostoma* species may be explained by a greater selective effect of hypermagnesian soil.

Introduction

New Caledonian ultramafic ranges are characterized by a variety of soil types supporting a diverse and endemic flora,[1] as is the case in many ultramafic ranges around the world.[2] The most common vegetation type found on ultramafic soils in New Caledonia consists of a secondary sclerophyllous shrubland, locally called 'maquis minier'. These formations are widespread, except in fire protected areas, which support localized pockets of rainforest.[3] Some types of 'maquis minier' are characterized by an open canopy dominated by the genus *Gymnostoma* (Casuarinaceae).[4–6] This genus has undergone considerable radiation in New Caledonia and is represented by eight endemic species; all possess a symbiotic association with nitrogen-fixing *Frankia* bacteria.[4] Recent molecular studies on the diversity of *Frankia* associated with *Gymnostoma* in New Caledonia[7,8] reveal that *Frankia* populations are genetically diverse. Nevertheless, the studies were unable to establish a strict relationship between the bacterial genotypes and the *Gymnostoma* species, as well as the edaphic requirements and geographic distribution of the *Frankia* populations.

The aim of this study was to define and compare the genetic diversity of *Frankia* populations associated with *Gymnostoma deplancheanum* and *G. chamaecyparis* growing on two types of ultramafic soils, and the floristic diversity of maquis containing these *Gymnostoma* species.

Materials and methods

Field surveys were conducted in *G. deplancheanum* maquis in the extreme south of the island and in *G. chamaecyparis* maquis at the base of the Tontouta valley (both in the southern ultramafic massif), and on the Boulinda massif on the west coast of the island (Fig. 1). *G. deplancheanum* maquis is widespread in the southern ultramafic massif on iron crust oxisol (*sol ferralitique ferritique gravillionaire ou cuirassé*[1]) and occurs south of an imaginary line between Nouméa and Ouinné. *G. chamaecyparis* maquis occurs on shallow slope deposits of hypermagnesian soil that develop on serpentinite outcrops at the base of ultramafic ranges situated to the north of the line between Noumea and Ouinné (Fig. 1). Maquis examined in the Tontouta valley is geographically close to *G. deplancheanum* maquis on iron crust oxisol, but shows no overlap. The maquis formations occurring at altitudes below 500 m a.s.l on the Boulinda massif are representative of this type of vegetation on isolated ultramafic ranges.

The soils supporting both types of maquis have distinct chemical properties (Table 1) despite sharing a common geological ancestry. They differ in nutrient availability and therefore have different effects on plant growth.[9,10] Both soils are very low in phosphorus, potassium and calcium. The iron crust oxisol contains elevated concentrations of manganese that may have toxic effects on plants owing to the acidic pH. Brown hypermagnesian soils are distinguishable from iron crust oxisols by their high magnesium level, which is potentially toxic to plants,[11] and by the strong Ca/Mg ratio imbalance, which reduces calcium uptake in plants.[12,13] Plants are less prone to manganese toxicity on hypermagnesian soils because of the elevated pH. In contrast to iron crust oxisols, hypermagnesian soils contain moderately high concentrations of nickel, which may have toxic effects on certain plants. However, the toxic effects of nickel uptake may be limited by the elevated concentrations of magnesium.[14–16]

The floristic survey of the three regions was carried out using phytosociological techniques and then examined together by factorial correspondence analysis (FCA)[17] using ADE-4, COA software[18] to determine the floristic associations belonging to each vegetation type. These species groups were then compared between vegetation types. The Shannon Weiner diversity index:

$$H' = -\sum_{i=1}^{S} P_i \log 2\, P_i$$

in which S is the floristic richness and $P_i = n_i/n$ is the relative importance of each species,[19] was calculated for each vegetation type from stem density measures recorded in representative 0.1 ha plots. The floristic evenness E, which describes the actual floristic diversity relative to the maximum possible diversity ($H_{\max} = \log S$), expressed as $H'/H_{\max}$, was also calculated for the 0.1 ha plots.

The analysis of *Frankia* genetic diversity incorporates several results from a previous study.[7] Both were conducted on nodules sampled from the two *Gymnostoma* species. This analysis consisted of isolating the DNA from a total of 65 nodules associated

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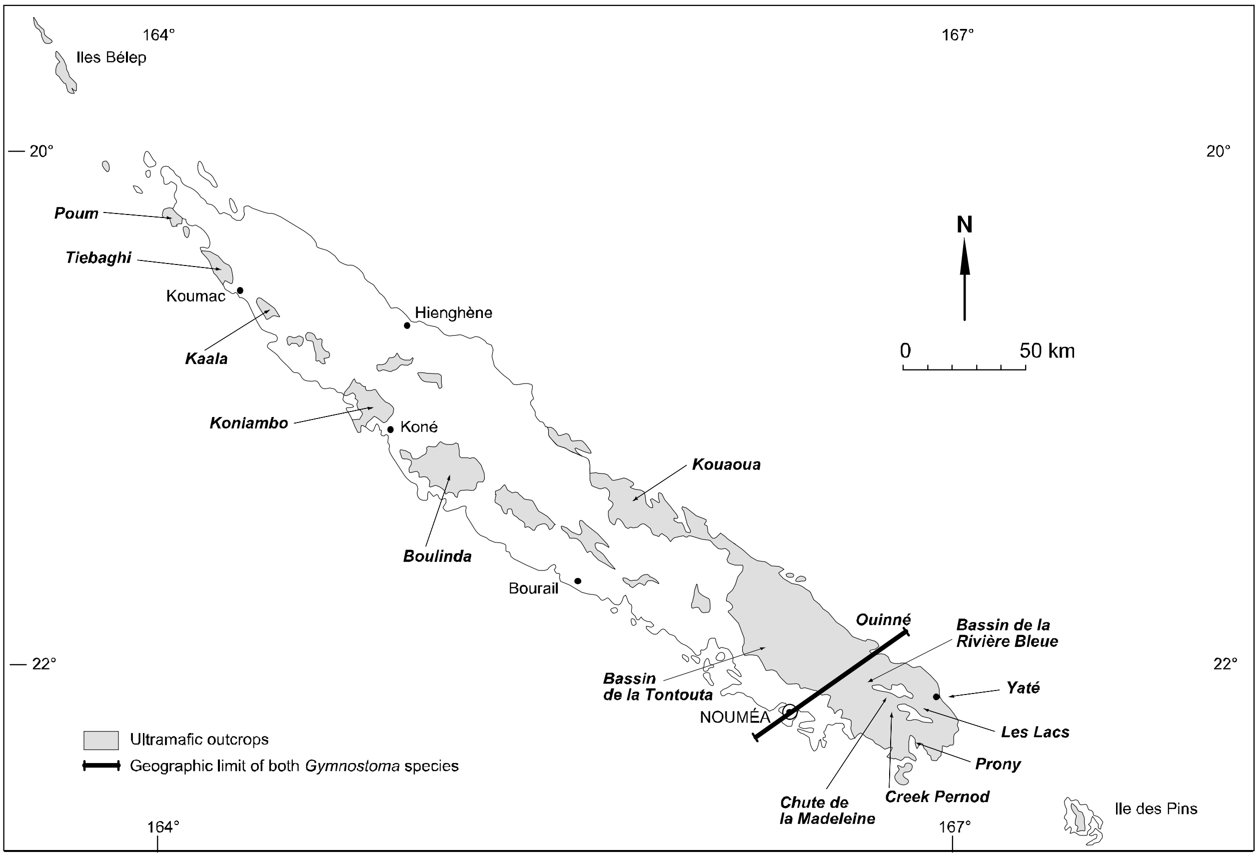

Fig. 1. Ultramafic outcrops in New Caledonia and geographic limit of both *Gymnostoma* species.

with *G. deplancheanum* and 90 nodules on *G. chamaecyparis*. Nodules were sampled on *G. deplancheanum* from six localities in the southern ultramafic massif. The nodules associated with *G. chamaecyparis* were sampled from seven localities found on six distinct ultramafic ranges (Fig. 1).

The DNA was extracted from one or two lobes of nodules that had been previously peeled. The extraction was carried out using the CTAB method.[20] The signature of this DNA was then magnified by PCR using primers found in the DNAr 16S and the DNAr 23S.[21,22] These primers encompass the intergenic space (IGS) and their amplification was carried out using the method described above.[20] The DNA extracted was digested using restriction enzymes (MspI, HaeIII) and the IGS groups developed along restriction profiles obtained for each enzyme. The methods used for DNA extraction, PCR amplification and *Frankia* characterization are described elsewhere.[20,7]

Results

Identification of floristic units

Three groups appear on the first and second axes of the FCA (Fig. 2). These groups correspond with the three regions studied. Floristic surveys conducted in *G. deplancheanum* maquis form a single unit. By contrast, the floristic surveys of *G. chamaecyparis* maquis separated into two distinct groups that correspond to different geographic zones.

The floristic records for *G. chamaecyparis* maquis of the Tontouta valley were analysed with floristic data of a previous phytosociological study of *G. deplancheanum* maquis in the south and *G. chamaecyparis* maquis on the Boulinda massif.[1]

The *G. deplancheanum* maquis flora includes species found on acidic soils — *Codia montana* (Cunoniaceae), *Styphelia veillonii* (Epacridaceae) and *Tristaniopsis Guillainii* (Myrtaceae) — and species restricted to iron crust oxisol — *Gardenia aubryi, Tarenna hexamera* (Rubiaceae) and *Pancheria confusa* (Cunoniaceae). *G. chamaecyparis* maquis is composed of species that are found mainly on hypermagnesian soils, such as *Hibbertia wagapensis* (Dilleniaceaee), *Xanthostemon multiflorus* (Myrtaceae), *Xylosma nervosum* (Flacourtiaceae), *Gahnia aspera* and *Scleria brownii* (Cyperaceae). The flora on hypermagnesian soil containing *G. chamaecyparis* in Tontouta valley is characterized by several locally restricted species: *Scaevola coccinea* (Goodeniaceae), *Cupaniopsis tontoutensis* (Sapindaceae), *Leptostylis petiolata* (Sapotaceae), *Syzygium tontoutensis* (Myrtaceae), *Tiegemopanax scopoliae* (Araliaceae), *Phyllanthus conjugatus* var. *conjugatus* (Euphorbiaceae) and *Homalium betulifolium* (Flacourtiaceae).

Table 1. Mean elemental composition (± standard error) of iron crust oxisols and brown hypermagnesian soils sampled from the root zone of *Gymnostoma deplancheanum* and *G. chamaecyparis*.

	Element									
Soil	pH	P (ppm)	Ca (%)	Mg (%)	K (%)	Na (%)	Mn (%)	Ni (%)	Fe (%)	SiO_2 (%)
Iron crust oxisol (*n* = 9)	4.63 ± 0.67	213 ± 111	<0.05	0.60 ± 0.50	<0.05	<0.05	0.23 ± 0.08	0.34 ± 0.14	42.5 ± 9.0	1.9 ± 2.9
Brown hypermagnesian soil (*n* = 9)	7.34 ± 0.39	89.5 ± 96.4	0.13 ± 0.11	10.88 ± 4.69	<0.05	<0.05	0.33 ± 0.15	0.69 ± 0.21	19.2 ± 9.2	36.34 ± 11.5

Table 2. Number of species (± standard error) of vegetation formations dominated by either *Gymnostoma deplancheanum* or *G. chamaecyparis*.

	Number of species		
Vegetation types	By plot (225 m²)	By plot (0.1 ha)	By floristic association
G. deplancheanum association (South)	25 ± 4 (*n* = 20)	58	72
G. chamaecyparis association (Tontouta, South)	38 ± 6 (*n* = 10)	64	103
G. chamaecyparis association (Boulinda)	28 ± 5 (*n* = 20)	64	89

Hypermagnesian soils supporting *G. chamaecyparis* maquis on the Boulinda Massif also contain several west coast hypermagnesian soil species, such as *Atractocarpus rotundifolius* (Rubiaceae), *Grevillea meisneri* (Proteaceae), *Phyllanthus montrouzieri* (Euphorbiaceae), *Xanthostemon ferrugineus* and *X. carlii* (Myrtaceae), and *Storckiella comptonii* (Caesalpiniaceae).

Comparison of floristic units

The floristic surveys conducted using a minimum area of 225 m² (0.1 ha) per plot, and the phytosociological analysis covering several hectares, indicate that the formations dominated by *G. chamaecyparis* contain more species than those dominated by *G. deplancheanum* (Table 2). The gregarious species, *Acacia spirorbis* (Mimosaceae), often abundant on the Boulinda range, generated a less apparent difference in floristic richness between *G. deplancheanum* and *G. chamaecyparis* maquis, mainly for 225 m² plots.

Floristic affinity and originality of the vegetation groups

The species recorded only in one of the three floristic associations are more abundant in *G. chamaecyparis* maquis than in *G. deplancheanum maquis* (Table 3). Furthermore, the *G. chamaecyparis* groups that are separated geographically, but found on the same soil type, share many species compared to *G. deplancheanum* and *G. chamaecyparis* maquis found in the southern ultramafic massif. These values include 11 ubiquitous species shared among the three floristic associations.

Floristic diversity

A comparison of values obtained by the Shannon Weiner diversity index and the floristic evenness (Table 4) based on woody species recorded for plots of 0.1 ha also indicate that *G. chamaecyparis* formations have a greater floristic diversity than *G. deplancheanum* maquis.

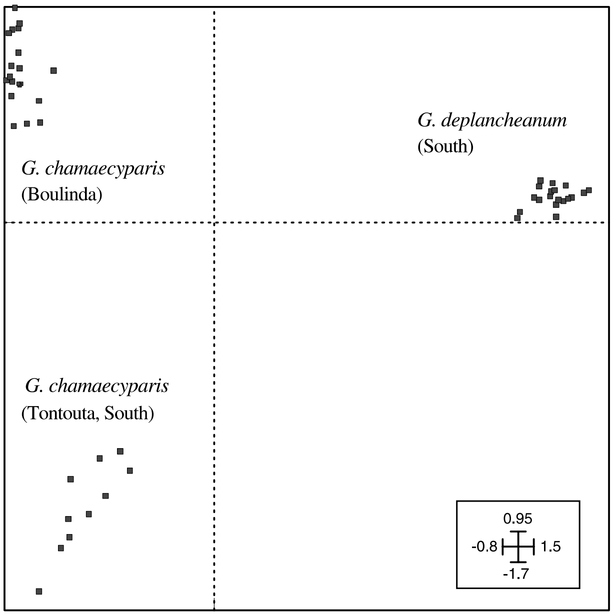

Fig. 2. Distribution of the three floristic groups on axes 1 and 2 of the FCA.

Table 3. Number of species found in only one, two or three floristic associations and number of shared species.

Floristic association	Species found in only 1, 2 or 3 floristic associations	Number of shared species
G. deplancheanum (South)	50	
G. chamaecyparis (Tontouta, South)	63	
G. chamaecyparis (Boulinda)	56	
G. deplancheanum (South) *G. chamaecyparis* (Tontouta, South)	9	20
G. chamaecyparis (Tontouta, South) *G. chamaecyparis* B(oulinda)	20	31
G. deplancheanum (South) *G. chamaecyparis* (Boulinda)	2	13
G. deplancheanum (South) *G. chamaecyparis* (Tontouta, South) *G. chamaecyparis* (Boulinda)	11	

Identification and distribution of DNA profiles of *Frankia*

Eleven different genetic patterns were identified in the DNA from *Frankia* from *Gymnostoma deplancheanum* nodules (Table 5). By contrast, seven distinct genetic types were identified in DNA isolated from *G. chamaecyparis* nodules (Table 6). Of the 15 genetic types identified, three were found in association with both species (4, 5 and 10).

Pattern 4 was the most common in nodules sampled from *Gymnostoma chamaecyparis* and was recorded for 87% of nodules analysed for all localities (Table 6). The six other types of *Frankia* identified each represented less than 3.5% of the DNA isolated from *Frankia* nodules sampled on *G. chamaecyparis.*

The *Frankia* associated with *G. deplancheanum* (Table 5) show three main genetic patterns, 5 (32%), 4 (25%) and 3 (18.5%), which together contribute 75% of the total *Frankia* genetic composition. This *Frankia* diversity is also composed of a type 6 (8%), a type 2 (6%) and six minor genetic patterns that each contribute 1.5% to 3% of the total *Frankia* genetic composition.

Eight *Frankia* genetic patterns were restricted to nodules on *Gymnostoma deplancheanum* (3, 2, 6, 18, 19, 20, 21, 22) and only three patterns of *Frankia* on *G. chamaecyparis* (7, 23, 24).

A total of 92% of *Frankia* in nodules sampled from *G. chamaecyparis* (4, 5, and 10) are also found on *G. deplancheanum* and 7.8% belong to other genetic patterns. By contrast, 58% of the *Frankia*

Table 4. Shannon Wiener index (*H'*) and floristic evenness (*E*) values for *Gymnostoma* formations.

	Woody species (0.1 ha)	
	H'	*E*
G. deplancheanum formation (South)	4.04	0.72
G. chamaecyparis formation (Tontouta, South)	4.68	0.80
G. chamaecyparis formation (Boulinda)	5.15	0.89

Table 5. The genetic diversity of *Frankia* associated with *Gymnostoma deplancheanum*.

Geographic locations	Number of nodules analysed	Intergenic space types										
		4	5	3	10	2	6	18	19	20	21	22
Creek Pernod	10	3	2	5								
La Madeleine	10	1	3	3						1	1	1
Les Lacs	6	2	3	1								
Rivière Bleue	12	2	5	1		2	2					
Prony	14	6	2	2	1		2		1			
Yaté	13	2	6			2	1	1	1			
Total	65	16	21	12	1	4	5	1	2	1	1	1
%		25	32	18.5	1.5	6	8	1.5	3	1.5	1.5	1.5

Table 6. The genetic diversity of *Frankia* associated with *Gymnostoma chamaecyparis*.

Geographic locations	Number of nodules analysed	Intergenic space types						
		4	5	10	7	13	23	24
Tontouta	31	23	2	1	1		2	2
Boulinda	19	19						
Kouaoua	10	7	1	1	1			
Koniambo	13	13						
Kaala	8	8						
Tiébaghi	6	6						
Poum	3	2				1		
Total	90	78	3	2	2	1	2	2
%		87	3.3	2.2	2.2	1.1	2.2	2.2

identified from nodules sampled on *G. deplancheanum* are also found on *G. chamaecyparis* and 41.5% belong to other genetic types (Table 7).

Discussion and conclusion

The vegetation associated with *Gymnostoma chamaecyparis* is floristically more rich than that associated with *G. deplancheanum*. This is particularly apparent when the floristic richness of different *Gymnostoma* vegetation groups found on the southern ultramafic massif are compared.

The vegetation associated with both *Gymnostoma* species is floristically different and possesses few species in common. *G. deplancheanum* maquis is characterized by a flora that has more species which are also found on other types of ultramafic oxisols or ferrallitic soils derived from acidic rocks. The maquis associated with *G. chamaecyparis* is composed of many specialized species that are mainly found on brown hypermagnesian soil.

By contrast, the findings of the DNA analysis indicate that the genetic types of *Frankia* DNA extracted from nodules on *Gymnostoma deplancheanum* are more numerous and less common than those originating from nodules on *G. chamaecyparis*. Moreover, the genetic types found in nodules of *G. deplancheanum* from six geographically close localities are more diverse than those found on *G. chamaecyparis* sampled from seven localities on six geographically isolated ultramafic massifs (Fig. 1).

Both *Gymnostoma* host species share two common genetic types of *Frankia* (4 and 5) and one that is not very abundant (type 10). Both type 4 and 5 are also generally found on other *Gymnostoma* species on a variety of soils and geographic regions in New Caledonia.[7] Nodules removed from *G. chamaecyparis* are largely dominated by genetic type 4. By contrast, type 5 is not very abundant in nodules found on *G. chamaecyparis.*

Table 7. The percentage of genetic types of *Frankia* that are common to or differ between both *Gymnostoma* species.

	Number of nodules	%
G. deplancheanum nodules		
Genetic types in common	38	58.5
Genetic types that differ	27	41.5
G. chamaecyparis nodules		
Genetic types in common	83	92.2
Genetic types that differ	7	7.8

Among the 11 genetic types of *Frankia* not shared between both vicariate species, types 3, 2 and 6 (identified from nodules on *Gymnostoma deplancheanum*) and type 13 (on *G. chamaecyparis*) are ubiquitous.[7]

The eight genetic types found exclusively in nodules of one of the two *Gymnostoma* species (types 7, 23, 24 on *Gymnostoma chamaecyparis* and types 18, 19, 20, 21 and 22 on *G. deplancheanum*) are relatively rare. Most of these genetic types were only identified once and it is therefore difficult to determine whether these types of *Frankia* are specialized and exclusively found on either host species.

One hypothesis to explain the difference in *Frankia* genetic diversity associated with the *Gymnostoma* species is the possible exclusion of a certain number of *Frankia* genetic types from *G. chamaecyparis* nodules, rather than the greater diversification of *Frankia* genetic types on *G. deplancheanum*. The absence of *Frankia* genetic types on *G. chamaecyparis* may be related to the edaphic constraints of hypermagnesian soil.

Edaphic properties of soils types supporting both *Gymnostoma* species may exert a selective role on certain genetic types of *Frankia.* However, contrary to the evolutionary differentiation of the flora into specialized species, it is possible that *Frankia* has not experienced the same evolution of soil-specialized strains since the origin of the ultramafic areas in the Oligocene.

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