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Biological *in situ* nitrogen fixation by an *Acacia* species reaches optimal rates on extremely contrasted soils

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ABSTRACT

Acacia spirorbis subsp. *spirorbis* Labill. is a legume tree that grows on calcareous, metalliferous and siliceous soils and is endemic to New Caledonia. The present study assesses the nitrogen-fixing potential of *A. spirorbis* in its contrasted natural environments.

Soil samples, the leaves of *A. spirorbis* and of co-occurring non-N_2-fixing reference plant species were collected from nine study sites across New Caledonia. Soil properties were analyzed by ICP-AES. Nitrogen (N) content and natural ^{15}N abundance (δ^{15}N) were measured in *A. spirorbis* and in reference plants. The percentage of N derived from the atmosphere (%Ndfa) fixed by *A. spirorbis* was also assessed and correlated with soil parameters.

Remarkably, mean N contents in *A. spirorbis* showed no significant differences whatever the soil categories ($20.2 \pm 3.5\,g\,kg^{-1}$) whereas major differences were found in N contents in the reference plants. The average δ^{15}N value of *A. spirorbis* was close to zero (+0.29‰), while that of the reference species ranged from −4.83 to +7.05‰. In eight of the nine sites studied, *A. spirorbis* %Ndfa was above 70%, regardless of the soil category. Unlike in the reference species, no statistical correlations were found between *A. spirorbis* N content and δ^{15}N abundance and soil parameters.

Despite the difficulty of using the δ^{15}N abundance method in such contrasted environments, the resulting data show that *A. spirorbis* has the highest N_2-fixing potential yet recorded in the genus *Acacia* and its nitrogen fixation is effective almost throughout its natural range.

1. Introduction

New Caledonia is a tropical archipelago located in the South Pacific Ocean, comprising the main island (*Grande Terre*), three smaller islands (Loyalty, Belep and the Isle of Pines) and many islets. The geological history of New Caledonia is complex and as a result, many kinds of bedrocks emerged about 37 Ma ago [1,2]. Climatic factors altered the bedrocks, resulting in the formation of different types of soils: ferralsols, acrisol, cambisol, leptosol, etc., and, in turn, in extremely contrasted soil properties [1,3]. Combined with topographical and climatic diversity, the geological history of New Caledonia means it is host to various ecosystems with a rich flora [1,4]. Over 3371 vascular plant species have been identified, of which 80% are endemic [5]. Ultramafic habitats likely shaped New Caledonia vegetation by promoting plant endemism and speciation [6]. Today, this exceptional flora is threatened by recurrent fires, extensive grazing, urbanization and mining [7,8] making New Caledonia a hotspot of biodiversity [9,10]. Minimizing the anthropic impact on the New Caledonian environment is a major challenge, and mining companies need to rehabilitate the soil after extracting the mineral resources [8]. Some potential soil rehabilitation species have already been tested including *Eucalyptus* and Australian *Acacias*, but none were able to adapt to the New Caledonian post-mining soils enriched in nickel [11]. Subsequently, endemic species were investigated, with a particular focus on those able to thrive despite poly-metal toxicity and, ideally, able to reintroduce nitrogen into the soil through rhizobia symbioses.

One notable candidate, *Acacia spirorbis* subsp. *spirorbis* Labill. (1825), grows over large areas and occurs naturally on all types of soils throughout the archipelago. This phyllodenous species of *Acacia* belongs to the Fabaceae family and the Caesalpinioideae subfamily in the clade of Australasian *Acacia* [12,13]. *Acacia spirorbis* is probably the result of natural long term dispersal from Australia [14] and displays invasive behavior in degraded ecosystems and deforested lands, which is a common feature of numerous Australian *Acacia* species [15–17]. Field observations have shown that *A. spirorbis* is spontaneously widespread in New Caledonia and grows in calcareous, ultramafic and

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volcano-sedimentary environments, with a pH ranging from 4 to 8, up to an elevation of 400 m asl., and on heavy metal or non-heavy metal enriched soils [18]. Recent field observations showed that *A. spirorbis* naturally establishes symbioses with arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (ECM) and nitrogen fixing bacteria [18,19]. These symbioses are presumed to play a key role in *A. spirorbis* adaptation to varied and extreme constraints. So far, only one study has been performed on the nitrogen fixing symbiotic status of *A. spirorbis,* which showed that the bacteria isolated from its root nodules were predominantly represented by *Bradyrhizobium* spp. strains [19]. However, no investigation has yet been undertaken of the potential key role of the N_2-fixing occurring with symbioses of *A. spirorbis* (shortened by N_2-fixation in the text) under such harsh soil conditions, especially in the N depleted soils found in ultramafic environments.

Assessing the N_2-fixation of a given legume tree that occurs naturally on extremely contrasted soils is challenging for two main reasons. The first is finding shared patterns in companion non-N_2-fixing reference species across the study sites. The second reason is coping with the marked variation in ^{15}N abundance in the reference species ($\delta^{15}N_{nf}$) that may occur across such contrasted environments.

The aim of the present study was thus to assess the N_2-fixing potential of *A. spirorbis* in various soil conditions, using the ^{15}N natural abundance method. This paper includes a brief description of the contrasted soil properties under which *A. spirorbis* occurs naturally, i.e. in metalliferous, calcareous and siliceous soils. The N contents and ^{15}N natural abundance ($\delta^{15}N$) of the legume tree and of non-N_2-fixing reference species were measured according to each site and each soil category concerned. The percentage of N derived from atmosphere (%Ndfa) by *A. spirorbis* was estimated in comparison with the companion reference species. Correlation analyses were performed between the soil properties of the different study sites and the nitrogen parameters.

2. Material and methods

2.1. Selection and description of the study sites

A total of nine sampling sites distributed across the main island and Loyalty Islands were chosen by superimposing the distribution map of *A. spirorbis* onto the geological map of the archipelago (Fig. 1). The sites selected were Easo (EAS), Pandanus 2 (PN2), Pindaï (PIN), Poum Malabou (PMB), Poum Silice (PMS), Serpent (SRP), Tiébaghi (TIE), Vertisol (VRT) and Wanaham (WAN), to ensure that the maximum range of soil conditions in New Caledonia were covered in our study.

2.2. Soil properties

The characteristics of the sampling sites are summarized in Table 1. Soils were identified using the WRB Classification (http://www.fao.org/3/a-i3794e.pdf). The soil samples were taken from the top 30 cm layer, where nodules are assumed to find the best conditions for their development [20]. Total concentrations of major and minor elements (Al, Ca, Co, Cr, Fe, K, Mg, Mn, Na, Ni, P and Si) in the soil were assessed at the ISO 9002-certified *Laboratoire des Moyens Analytiques* (LAMA) in Noumea IRD Center (New Caledonia) by ICP-AES after alkaline fusion. Organic carbon (C) and nitrogen (N) contents were determined, following the Walkley-Black and Kjeldahl methods respectively, as described by Perrier et al. [21].

The soils of the nine study sites can be grouped in three contrasted soil types, metalliferous, calcareous and siliceous according to their chemical composition (Table 2) as revealed by principal component analysis (PCA) (Fig. S1). The metalliferous soils (at the Pindaï, Pandanus 2 and Tiébaghi sites) contain significantly higher concentrations of metallic elements than the siliceous and organic soil types (all sites combined), in particular Co, Cr, Fe, and Ni, which are on average 10, 40, 3 and 40 times higher, respectively (Table 2). The calcareous soils (at Easo and Wanek sites) have the highest concentrations of C, N and P elements respectively, 3, 8 and 40 times higher on average than those obtained in the two other soil types (Table 2). The siliceous soils (at Poum Malabou, Poum Silice, Serpent and Vertisol sites) are characterized by the highest Si contents, on average 2 times higher than those of metalliferous or calcareous soils (Table 2).

The total N contents of soils (N_{soil}) differed significantly ($P < 0.001$) with the site, with the lowest N_{soil} values (less than 500 mg kg^{-1}) measured in Pandanus 2, Tiébaghi and Poum silice sites and the highest in Easo (21,817 mg kg^{-1}) (Table S1). Although Easo and Wanaham presented contrasted soil N contents, calcareous soils had higher N_{soil} values than metalliferous or siliceous soils, averaging 1338 and 1182 mg kg^{-1} respectively. The soil ^{15}N abundances ($\delta^{15}N_{soil}$) were all positive and differed significantly according to the site ($P < 0.001$), ranging from +3.04‰ in Serpent site up to +11.0‰ in Pandanus 2 site (Table S1).

2.3. Sampling method and collection of plant material

In each of the nine selected sites, we collected all the leaves from three *A. spirorbis* trees growing at a distance of at least 50 m apart and leaves from non-N_2-fixing reference tree or shrub species found within a 2–4 m radius from the base of the trunk of each *A. spirorbis* tree sampled. The leaves sampled from each individual tree were reduced to form representative subsamples each weighing about 10 g (fresh weight). Seven non-N_2-fixing reference species were sampled across the nine sites, namely *Dodonaea viscosa* Jacq., *Melaleuca quinquenervia* (Cav.) S.T. Blake, *Morinda citrifolia* L., *Psychotria nummularoides* (Guillaumin), *Sannantha* spp., *Scaevola* sp. and *Wikstroemia indica* (L.) C.A. Mey., confirmed by referring to the botanical collection at the IRD Herbarium in Noumea. These plant species were selected (i) according to their representativeness and abundance in each study site and (ii) because they occurred in a maximum of study sites. Each of the reference species was present in at least two sites, but none was present in all nine study sites (Table 1). However, *Wikstroemia indica* was present in the three soil types and in five of the nine study sites (Table 1), and, like *A. spirorbis*, is one of the rare ubiquitous plant species found in the three major soil types in New Caledonia. *Melaleuca quinquenervia* (Myrtaceae), an ectomycorrhizal tree species [22] like *A. spirorbis*, was also found in five of the nine study sites but only in two of the three soil types, the metalliferous and siliceous ones (Table 1). The leaves and soil samples were oven dried at 50 °C for 48 h and ground into a fine powder with a sample mill (Foss Cyclotec 1093). Next, 5–10 mg (dry weight) of each sample were transferred into tin capsules before total N contents and $\delta^{15}N$ were determined using a CN analyzer in tandem with a continuous flow isotope ratio mass spectrometer (Europa ANACA-GSL, PDZ Europa Ltd, Sandbach, UK) at the UC Davis (University of California, Davis) Stable Isotope Facility for leaf samples. The $\delta^{15}N$ in the soil samples was assessed at the *Laboratoire des Moyens Analytiques* (LAMA) of the Noumea IRD Center (New Caledonia), on a CHN analyzer Sercon Integra 2, combining a Sercon 20–22 Stable Isotope Ratio Mass Spectrometer (IRMS) and an SL elemental analyzer.

2.4. Determination of the proportion of nitrogen derived from atmospheric N_2

The percentage of nitrogen derived from atmospheric N_2 (%Ndfa) was calculated according to the following equation [23]:

$$\%Ndfa = (\delta^{15}N_{nf} - \delta^{15}N_f) / (\delta^{15}N_{nf} - \beta) \times 100$$

Where $\delta^{15}N_{nf}$ is the natural ^{15}N abundance in the non-N_2-fixing reference species, $\delta^{15}N_f$ is the natural ^{15}N abundance in *A. spirorbis* and β, the $\delta^{15}N$ measured in leaves of nodulated *A. spirorbis* growing in a N-free nutrient medium that fixed 100% of atmospheric N_2. In this study, the average β was −0.937‰ (standard error = ±0.37‰). The β value was calculated from three *A. spirorbis* seedlings inoculated with a