



Short communication

Soil *Bradyrhizobium* population response to invasion of a natural *Quercus suber* forest by the introduced nitrogen-fixing tree *Acacia mearnsii* in El Kala National Park, Algeria



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ABSTRACT

We analyzed the diversity and identity of the rhizobial populations nodulating an invasive Australian legume tree *Acacia mearnsii* in a natural *Quercus suber* forest in the El Kala National Park, Algeria. Soils from three different forest plots corresponding to non invaded original *Q. suber* stand, partially invaded by *A. mearnsii*, and totally invaded (monodominant) *A. mearnsii* stand were used to trap nodulating bacteria with the same tree species. Symbiotic nitrogen-fixing bacteria were isolated from root nodules and characterized by sequencing of the internal transcribed spacer region, then submitted to phylogenetic analyses. A total of 67 isolates was obtained, representing the 3 different forest plots, all renodulating *A. mearnsii* in monoxenic conditions. Phylogenetic analyses showed that all isolates belong to different *Bradyrhizobium* lineages, according to each of the three locations with little intermixing between forest plots. These results illustrate the adaptation of nodulating *Bradyrhizobium* populations to the new soil conditions induced by invasion. This symbiotic adaptability is presumed to be a key factor of the invasive character of this tree species.

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Black wattle (*Acacia mearnsii* De Wild.) is an Australia native species of the Fabaceae family that belongs to the subfamily Mimosoideae. Since the 19th century, *A. mearnsii* has been exported, as a source of tannin bark for leather industry, in Africa, Brazil and India. It is now distributed in many countries and considered invasive (<http://www.gisin.org/DH.php?WC=/WS/GISIN/home.html>). *A. mearnsii* is known to be nodulated by slow growing species of the genus *Bradyrhizobium* (Dreyfus and Dommergues, 1981; Turk and Keyser, 1992; Lafay and Burdon, 2001).

In Algeria, the El Kala National Park was created in 1983 and recognized as a Biosphere Reserve by the UNESCO in 1990. The area is known for its rich biodiversity. Among forest ecosystems are *Quercus suber* stands that are threatened by invading *A. mearnsii* spontaneously colonizing these stands from old eucalypts-*A. mearnsii* mixed plantations. The progressive settlement of *A. mearnsii* has a dramatic effect on the initial *Q. suber* stands, leading to the irreversible disappearance of *Q. suber* and its natural

floristic associates, ending in almost pure stands of *A. mearnsii*. In a preceding work, we showed that this invasion had a strong deleterious effect on soil chemical characteristics, on soil microbial functions and ectomycorrhizal communities associated to the early growth capacities of *Q. suber* seedlings (Boudiaf et al., 2013). In this work, we aim at understanding how this *A. mearnsii* deals with its nitrogen fixing symbiotic partners from soils taken along an invasiveness gradient, from a natural *Q. suber* stand to a partially invaded one and then to a pure spontaneous stand of *A. mearnsii*.

The experimental area, soils sampling and characteristics were fully described in Boudiaf et al. (2013). Briefly, they consisted of pooled soil samples taken along an invasiveness gradient, from a natural *Q. suber* stand (S1) to a partially and spontaneously invaded one (S2) and then to a pure and also spontaneous stand of *A. mearnsii* (S3). To our knowledge, no rhizobial inoculation of any tree has ever been made in the whole area. The soil samples (S1, S2 and S3) were used to obtain *A. mearnsii* – nodulating rhizobia by soil trapping. Young seedlings of *A. mearnsii*, germinated in agar plates, were inoculated with 60 g of soil in top of a pot filled with sterile sand-loam and grown in Mediterranean greenhouse with 5 replicates of 2 plants for each soil. After plant nodulation, 12

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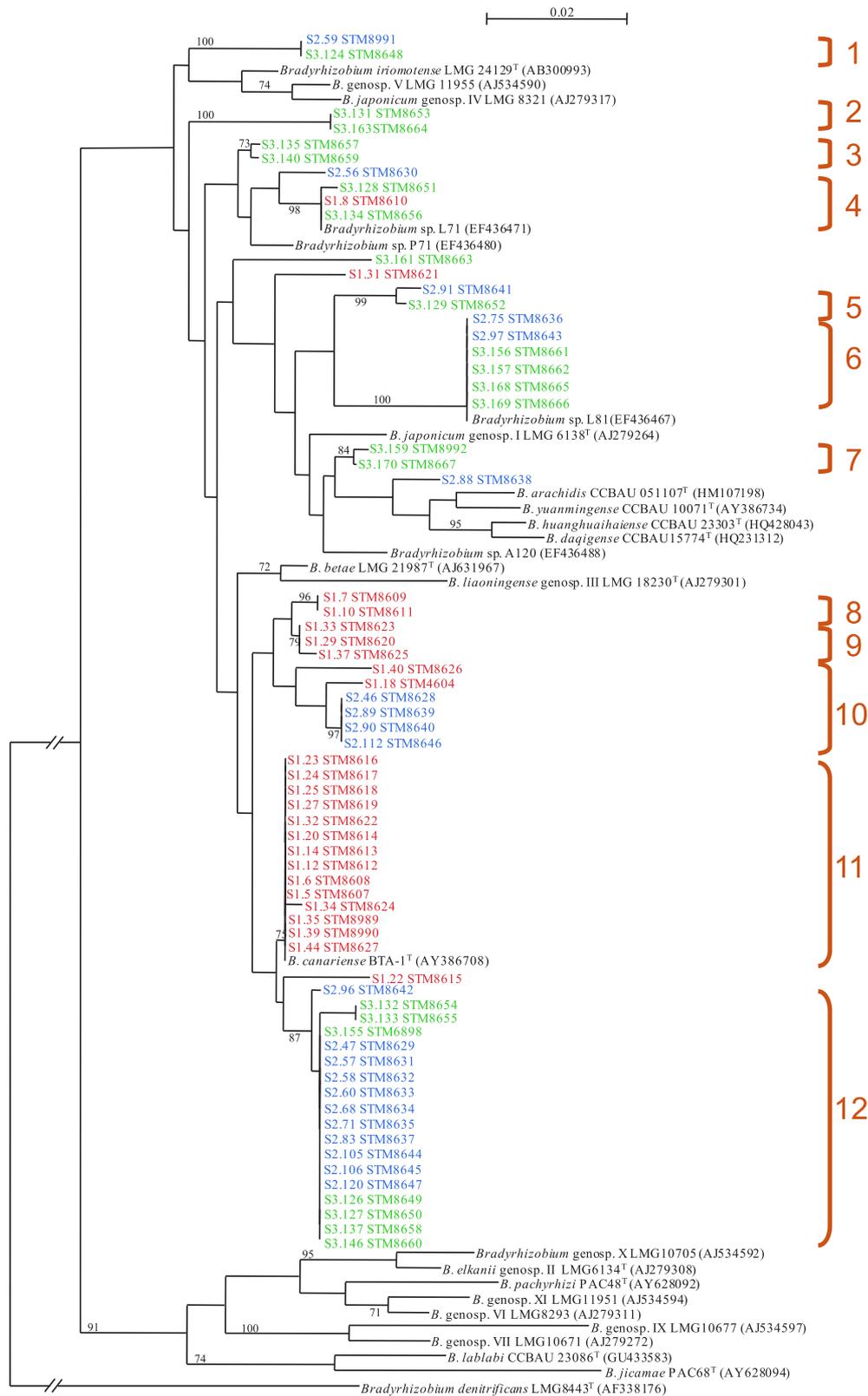


Fig. 1. Phylogenetic tree based on analysis of partial ITS sequences of 67 *Acacia mearnsii* isolates and related bradyrhizobia strains, determined by using the neighbor-joining method and rooted with *Bradyrhizobium denitrificans*. Numerical values at the branching points are the bootstrap values of 100 replications. Only bootstrap values higher than 70% are given. The database accession numbers are indicated in parentheses. The type strains are indicated with the character "T". S1: natural *Quercus suber* stand, S2: mixed (invaded) *Q. suber*/*Acacia mearnsii* stand, S3: pure, totally invaded, *A. mearnsii* stand.

nodules were collected on each plant for bacterial isolation. The isolation procedure and monoxenic renodulation assays were made according to Perrineau et al. (2011). The re-nodulating strains were investigated by PCR amplification and sequencing of the 16S–23S ITS region. A loopful of rhizobial cells was suspended in 50 μ l of sterile water and 2 μ l of this suspension was used as template DNA for subsequent PCR amplification. The PCR conditions, DNA sequencing, sequence analysis and phylogenetic tree construction were as described in Le Roux et al. (2009), except that sequencing was performed by Genoscreen (Lille, France). The partial 16S–23S ITS sequences were deposited in GenBank sequence database under the following accession numbers: JX311553–JX311619. A factorial correspondence analysis (FCA) was performed to visualize the relationships between the number of strains within each cluster from the phylogenetic analysis of ITS sequences and soil type using XLSTAT software package (version 2010.5.04, Addinsoft, Paris, France, <http://www.xlstat.com>).

All soils were acidic which is the natural and general condition of *Q. suber* stands (Robert et al., 1996). Their characteristics (pH, total C, total N, total and available P) are commented in Boudiaf et al. (2013) with the most acidic site (S3) being the most invaded one and total nitrogen values increasing with the invasion (S2 site) but decreasing in the completely invaded stand S3. After trapping, a total of 67 isolates were obtained among which 24 (STM collection number 4824, 8989, 8990 and 8607–8627), were from S1, 21 (STM collection number 8991 and 8628–8646) from S2, and 22 (STM collection number 6898, 8992 and 8647–8667) from S3. All isolates belong to the genus *Bradyrhizobium* and successfully nodulated *A. mearnsii* in monoxenic conditions. The ITS sequence length ranged from 646 to 739 bp. In the alignment matrix obtained (data not shown), 2 sequences (S2.91STM8641 and S3.129STM8652) displayed the highest number of indels (i.e. 5) respectively 6, 12, 2, 53 and 13 bp long. Others indels are much shorter and rare, with the exception of a 15 bp insertion that affects 18 sequences. The phylogenetic tree is illustrated in Fig. 1. In the natural *Q. suber* stand, most of S1 strains (14 isolates) are grouped (cluster 11) with *Bradyrhizobium canariense* BTA-1 bv. genistearum. Two other clusters (8 and 9) include isolates with no close type strain of *Bradyrhizobium*. Some S1 isolates are punctually distributed among diverse populations including S2 and S3. In the S3 and S2 situations, the populations are distinct from S1, more diversified and intermixed, grouping on one hand with *Bradyrhizobium japonicum* LMG 6138, on the other hand with *Bradyrhizobium* sp. L81 from *Acacia longifolia*. A third group of 18 isolates (11 isolates from S2 and 7 from S3), clustered together without reference species, the closest species being *B. canariense* BTA-1 bv. genistearum. Using FCA, Fig. 2 shows that *A. mearnsii* drastically impacts on the soil *Bradyrhizobium* populations, along the F1 axis that represent 80.6% of the variability, isolating S1 strains, with S2 and S3 populations being closer to each others.

As reminded by Richardson et al. (2000) many introduced plant species rely on mutualisms in their new habitats to overcome barriers to establishment and to eventually become invasive. Concerning nitrogen-fixation, nodulating bacteria are extremely widespread, but there are big differences among plant genera with respect to the specificity of their partners. According to Rodriguez-Echeverria et al. (2011), who examined 8 different species of Australian acacias, there is no clear difference in *Bradyrhizobium* diversity between invasive and non-invasive Australian acacias. *Bradyrhizobium* is the most dominant nodulating partner of these acacias in native or non-native regions and we showed this was the case in Algeria. These strains grouped into populations that seem specific for the most part in S1 isolates and intermixed in the S3 and S2 situations. No populations were found within *Bradyrhizobium elkanii* clade, a species detected with several Australian acacias like

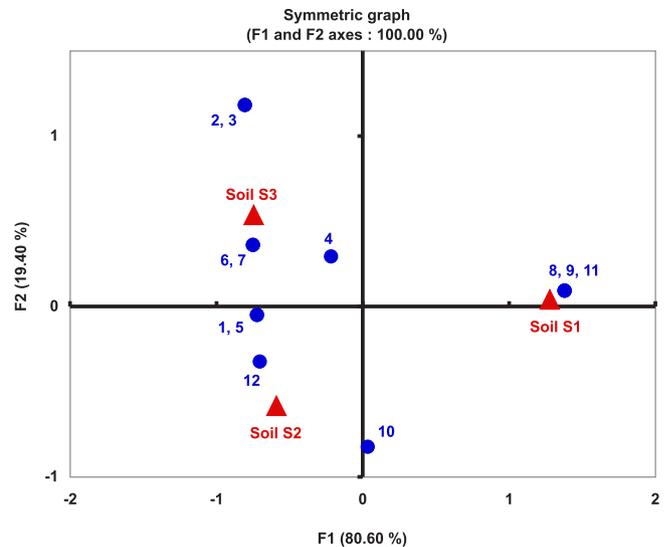


Fig. 2. First factorial plane projection of the factorial correspondence analysis between the 12 bacterial phylogenetic clusters defined in Fig. 1 (blue circles) and the three soil origins (red triangles), S1: natural *Quercus suber* stand, S2: mixed (invaded) *Q. suber*/*Acacia mearnsii* stand, S3: pure, totally invaded, *A. mearnsii* stand. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

A. mearnsii, *Acacia dealbata* (Rodriguez-Echeverria et al., 2011), *Acacia mangium* (André et al., 2005) or *Acacia cincinnata* (Rodriguez-Echeverria, 2010). This result is consistent with the data of Lafay and Burdon (2001) who confirmed that *B. japonicum* is the most representative species with 97% of the isolated rhizobia in Australian acacias, although strains of *B. elkanii* were rarely found. The origin of these Algerian strains could be probably more precisely elucidated using multiloci sequencing of both housekeeping and symbiotic genes and including Australian strains in the analysis. The source of rhizobial symbionts for invasive acacias is ecologically relevant not only to understand the process of invasion but also to assess the impact on the native plant and soil communities (Rodriguez-Echeverria et al., 2011). The rhizobial resource must not be a limiting factor and highly promiscuous legumes should be better colonizers than those with a narrow range of symbiotic specificity. Australian acacias, at least those that have been checked among the ca 1000 native species, appear to be nodulated predominantly by the genus *Bradyrhizobium* (Birnbaum et al., 2012). Inoculations practices, particularly with sympatric (Australian) strains have often been used with the most economically important acacias (e.g. *A. mangium*) (Galiana et al., 1998) but they never transformed their host into an invasive plant. The question of the relationships between bradyrhizobial communities and the invasion success of several acacias, in their native country (but not environment), has been recently raised by Birnbaum et al. (2012), who showed that the invasive potential could not be only reduced to mutualistic relationships. Together with the recent work, published on the same environment but on the threatened local trees species *Q. suber* by Boudiaf et al. (2013), our study on the invasive *A. mearnsii* shows that microbial communities of both plants are dramatically impacted by invasion probably in response to the new soil conditions (acidification, C/N ratio, P content) observed in the invaded sites.

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