



Effect of anthropisation and revegetation efforts on soil bacterial community structure in Terga sandpit, Algeria

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Abstract

Impact of anthropisation and revegetation on soil microbial diversity was studied by characterizing bacterial community structure in a sandpit located in Terga (Algeria). Soil samples were collected from several sites: an undisturbed forest area with pristine vegetation, a site revegetated by the National Forest Services in 1998, a newly revegetated site by the introduction of two tree species (*Tetraclinis articulata* and *Schinus terebinthifolius*) associated, or not, with two legume species (*Retama monosperma* and *Lotus creticus*). Samples were collected from bulk and rhizospheric soil compartments during an 18 month-period. Soil bacterial community structure was characterized by rRNA Intergenic Spacer Analysis (RISA) and statistical analysis. Concerning revegetated sites, soil physicochemical analysis was performed just after plantation and after 18 months. The plant effects on bacterial community structure differed among sites and plant species. In pristine forest, bacterial community structures of bulk and rhizospheric soils are most similar, both in time and whatever the cover plant species. In the recolonized site, no temporal change was observed, but plant, may impact bacterial community structure in rhizosphere. '*T. articulata*' impacted bacterial community structure all along the temporal scale. Opposite, no significant effect of *S. terebinthifolius* was detected. A correlation between soil parameters and bacterial community structure was observed, suggesting that plant may drive bacterial community structure by providing nutrients and modifying soil physicochemical parameters. These results evidence the dynamics of microbial communities in response to revegetation efforts; registering microbial community evolution in time may help to select plant species with biological soil quality enhancing potential.

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Introduction

Human activities like mining or quarrying generate soil degradation (soil characteristics and functions) that rapidly and drastically affects biogeochemical cycles (Citeau *et al.*, 2008; Lehmann and Stahr, 2007) and causes disturbances and damages to ecosystem processes (Jaffré and Rigault, 1991). Ecological restoration has become an important part of sustainable development (Sheoram *et al.*, 2010). Specifically, revegetation is one of the most widely used practices to restore disturbed ecosystems (De Mei and Di Mauro, 2006; Duponnois *et al.*, 2013). Revegetation programs include pioneer plants adapted to these drastic environments, among which those developing symbioses with microorganisms (Franco *et al.*, 1994; Pelletier and Esterle, 1995; Brennan, 1996; Wilson and Hanlon, 2012). The latter include (i) nitrogen-fixing bacteria, enabling symbiotic plant to grow in nitrogen poor soil and contributing to N input into the ecosystem and (ii) mycorrhizal fungi involved in plant nutrition improvement, especially Phosphorus uptake (Requena *et al.*, 2001). Symbioses were included in revegetation programs in several anthropic ecosystems like nickel mines in New Caledonia (Jaffré *et al.*, 2001, Herrera *et al.*, 2007), sand dune stabilization in Senegal (Diagne *et al.*, 2013) and degraded sites in the Mediterranean (Brunel *et al.*, 2007). *Acacia* species were used for spoiled soil revegetation, and were introduced for soil rehabilitation in many countries, particularly in North Africa since 1870 (Poynton, 2009; Carruthers *et al.*, 2011). Symbioses increase plant survival and facilitate ecological succession (Lugo, 1997). However, despite research efforts, determining optimal strategy for soil revegetation is still hindered by limited knowledge on environmental processes and ecosystem functioning. Soil microorganisms play central roles in environmental processes such as mineral and organic matter recycling (soil fertility) (Swift *et al.*, 1998), nutrient and water availability, carbon and nitrogen cycles (Hattenschwiler *et al.*, 2005).

The extensive exploitation of Terga dune (NW Algeria) during the last decades induced a continuous and drastic decrease of vegetation cover. In recent years, several revegetation efforts have been performed, attempting to directly introduce pioneer plant candidates, without considering any local parameter, including soil biology (Bouazza Marouf *et al.*, 2015). However, studying bacterial community evolution could provide news insights on sandpit restoration and soil functioning, useful information for revegetation strategy success. Therefore, the aim of the present work was to investigate the impact of anthropisation and revegetation efforts on soil functioning. For this purpose, we followed the evolution of bacterial community, using RISA fingerprinting (Ranjard *et al.*, 2000b; Acinas *et al.*, 1999; Borneman and Triplett, 1997; Felske and Akkermans, 1998) in pristine and disturbed environments of the sandpit of Terga (Algeria): (1) a native forest, (2) a replanted site by National Forests Services in 1998 (“Recolonized Sand spoils”), (3) a new “Revegetated Sand spoils trial” initiated at the beginning of this work.

Materials and methods

Sites and sampling

Soil samples were collected from a sandpit of Terga fore dune, which is located on the central part of the Témouchent region, along West Algerian coast. The dune covers an area of about 120 ha (Fig. 1), 45% of which is being exploited for sand production. This sandpit is located along the estuary of Wadi El Maleh (35°26'24.35"N, 1°13'35.20"O) (Fig. 1). Three contrasting study sites were chosen in the sandpit area (Table 1). One corresponded to the revegetated zone set up in April 2012 by planting four plants species: two tree species (*Schinus terebenthifolius* and *Tetraclinis articulata*) associated, or not, with 2 legume plants (*Retama monosperma* and / or *Lotus creticus*). The experimental design is depicted in Fig. 1. The second site was located in the zone revegetated by the forest services in 1998 (Recolonized sand spoils). The third one was chosen inside an undisturbed forest area with pristine vegetation. For the two latter sites, only legume plants were considered in the study.

Table 1. Description and name of soil samples in Terga sandpit.

Nomenclature	Soil type	Plant
SB	Revegetated Sand spoils	Bare soil
S1	Revegetated Sand spoils	<i>Tetraclinis articulata</i>
S2	Revegetated Sand spoils	<i>Tetraclinis articulata</i> and <i>Retama monosperma</i>
S3	Revegetated Sand spoils	<i>Tetraclinis articulata</i> and <i>Lotus creticus</i>
S4	Revegetated Sand spoils	<i>Tetraclinis articulata</i> , <i>Lotus creticus</i> and <i>Retama monosperma</i>
S5	Revegetated Sand spoils	<i>Schinus terebinthifolius</i>
S6	Revegetated Sand spoils	<i>Schinus terebinthifolius</i> and <i>Retama monosperma</i>
S7	Revegetated Sand spoils	<i>Schinus terebinthifolius</i> and <i>Lotus creticus</i>
S8	Revegetated Sand spoils	<i>Schinus terebinthifolius</i> , <i>Lotus creticus</i> and <i>Retama monosperma</i>
P1	Pristine vegetation	Bare soil
P2	Pristine vegetation	<i>Retama monosperma</i>
P3	Pristine vegetation	<i>Lotus creticus</i>
R1	Recolonized Sand spoils	Bare soil
R2	Recolonized Sand spoils	<i>Retama monosperma</i>
R3	Recolonized Sand spoils	<i>Lotus creticus</i>

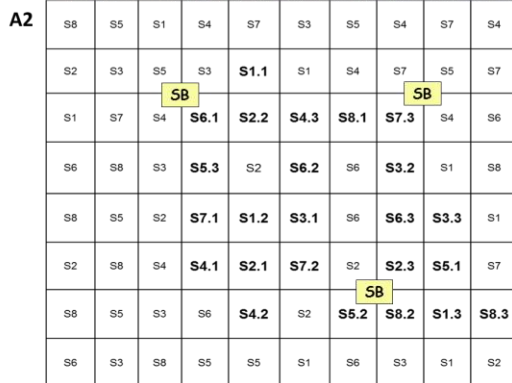


Fig. 1. The study site.

A1 Location of sampling sites in the Terga Sandpit. <https://www.google.fr/maps?hl=fr>

A2 Schematic diagram of the experimental design. Bold characters correspond to rhizospheric samples. The squares correspond to bulk soil samples. See Table 1 for treatment nomenclature.

To follow the differential effect of plants in time: (1) In revegetated site, three temporal samplings were

performed at 15 days, 12 and 18 months after plantation. The rhizospheric soils were sampled around the roots without destroying plants. (2) In the two older sites, two temporal samplings were performed at an initial time (T0) and after 12 months. The rhizospheric soils were sampled with roots. In each site, bare soils were sampled at 0-10 cm depth from the surface after eliminating first soil layer. All plant- and bare- soil samples were performed in triplicates. After sampling, the soils were dried for 24 hours at room temperature, and then sieved at 2 mm.

In revegetated site, soil physicochemical analysis (texture, pH, Electrical Conductivity, Total Nitrogen, Total Limestone, Organic Matter and Available Phosphorus) was performed by agronomic laboratory of FERTIAL Company and National Institute of Agronomic Research of Algeria (INRA) using standard procedures. Soil was sampled in triplicates, at the beginning of plantation (Bare soil) and after 18 months. Each set of triplicate samples was pooled to create a single composite sample. In order to evaluate physicochemical changes, principal component analysis (PCA) was performed using R software (R Development Core Team, 2011) and the package ADE4TkGUI (Thioulouse and Dray, 2007).

DNA extraction

DNA was extracted from 10g of each soil sample using an Ultra Clean Mega Soil DNA kit (Mobio, CA) according to the manufacturer instructions. Soil DNA was then further purified with Nucleo Trap® nucleic acid and protein purification Kit (Macherey-Nagel) or the Power Clean® DNA Clean-Up Kit (Mobio), depending on the DNA purity. DNA concentrations were determined on 1 µl DNA preparation with the Qubit BR kit or the high sensitivity kit (Invitrogen, Fisher scientific, Illkirch, France) on Qubit Fluorometer (Invitrogen), depending on DNA concentrations.

Inter-Genic Spacer (IGS) Amplification

The intergenic spacer region (IGS) between the small and large subunit rDNA gene was amplified, using 1 ng DNA template and the universal primers: SD-Bact-1522-bS-20 and LD-Bact-132-aA-18 (Ranjard *et al.*, 2000a). Amplification reaction was performed in a final volume of 25 µl containing 0.2 µM of each primer, 1.3 M betaine and 1X Cesium polymerase Klentaq AC LA PCR Kit reagent (DNA Polymerase Technology, St. Louis, Missouri). Amplification was performed as follows: preheating at 95°C for 2min, followed by 30 cycles: denaturation at 94°C for 50 s, annealing at 55°C for 50 s and extension at 68°C for 2min. After a final extension step at 68°C for 5 min the reaction is cooled and kept at 10°C. In order to eliminate inhibitors, PCR products were purified with the Nucleo Spin Gel and PCR Clean-up kit (Macherey-nagel). Amplification profiles were checked on 1% agarose gel.

Ribosomal Intergenic Spacer Analysis (RISA) and Statistical analysis

RISA was performed in a 2100 Bioanalyzer (Agilent Technologies, USA), using High Sensitivity DNA chips according to the manufacturer's recommendations. The 2100 expert Agilent software determines peak sizes and areas in reference to both internal size standards included in each test sample and an external ladder and then converts fluorescence data into electrophoregrams. The data are exported in a CSV format and then imported into the RISA Aligner program (Navarro *et al.*, 2015) for alignment and normalization.

To compare soil microbial communities, Between Component Analysis (BCA) of their RISA patterns was performed using R software (R Core Team (2015) and the package ADE4TkGUI (Thioulouse and Dray, 2007).

Relationships between bacterial community structure and soil physicochemical features

In order to analyze the relationships between soil physicochemical characteristics and bacterial community structures, we performed a coinertia analysis, a standard multivariate analysis that describes the relationships between two data tables (Doledec and Chessel, 1994; Dray *et al.*, 2003). In order to proceed to coinertia analysis, two principal component analyses (PCA) were performed: the first one to describe the genetic structure of bacterial communities and the second to describe the soil physicochemical characteristics. PCA, co-inertia analysis and Monte Carlo test, used to check the significance of the analysis, were performed using the Ade4TkGUI software (Thioulouse and Dray, 2007).

Results**Soil characteristics**

Bare and rhizospheric soils were sampled at different times during a 18 month period in order to evaluate plant species and temporal scale effects on bacterial community structure. Soil analysis (Table 2) indicates that all soils exhibit a sandy texture with about 94% sand, with good internal drainage and low water- and fertilizer- retention capacity. Principal component analysis (PCA) showed differences in soil physicochemical characteristics at the beginning of plantation and after 18 months (Fig. S1). The first axis, explaining 61.9% of the variance, separated bulk soils and rhizospheric soils under *T. articulata* or *S. terebinthifolius* from the others plant combinations. This separation was correlated to high nitrogen and phosphorus contents and low pH (Table 2). Organic matter and electric conductivity remain stable in all soils, averaging 0.1 and 0.19 respectively. Opposite, limestone rate increases when plants are present (Table 2).

Table 2. Soil characteristics at the initial stage and after 18 months of plantation.

Soils	Texture (%)			Conductivity 1:5(ms.cm ⁻¹)	pH	Total CaCO3 (%)	Organicmatter (%)	Available phosphorus (ppm)	Total nitrogen (%)
	Sand	Limon	Clay						
SB-0	94	4	2	0.12	8.80	28.60	0.007	6.30	0.02
SB-18	95	3	2	0.19	7.87	29.33	0.005	6.20	0.02
S1-18	95	3	2	0.20	7.90	33.80	0.005	6.60	0.02
S2-18	94	4	2	0.20	7.30	41.00	0.010	9.00	0.03
S3-18	93	4	3	0.20	7.20	39.30	0.001	9.80	0.04
S4-18	94	4	2	0.19	7.30	40.20	0.006	9.20	0.03
S5-18	94	4	2	0.17	7.50	40.00	0.008	7.00	0.02
S6-18	95	2	5	0.25	6.00	37.00	0.010	9.80	0.04
S7-18	94	3	3	0.21	6.80	37.40	0.010	11.00	0.05
S8-18	94	3	3	0.22	7.20	34.00	0.009	10.80	0.03

Note: 0 and 18 after sample name/code correspond to time sampling.

Spatio-temporal evolution of soil bacterial community structure

The impact of plant cover restoration on soil bacterial community structure was performed by RISA fingerprinting method followed by BCA pattern analysis.

In a first step, the soil bacterial community structures were compared in the two oldest sites: pristine and recolonized. Bacterial structures differed in the two sites. In pristine soils, bacterial community structures were rather similar whatever the soil type (rhizospheric or bare) and the sampling time as shown by BC Aanalysis (Fig. 2A1). Opposite, in the recolonized site, a major impact of plant on soil bacterial diversity was observed (Fig. 2A2). The first axis of the BCA explains 44.2 % of the variance and shows a strong separation between bare and rhizospheric soils. However, the plant type has less impact. In both sites, no obvious temporal effect on the bacterial community structure could be established.

Opposite, a temporal effect on bacterial community structure was observed in soil under *T. articulata* whatever alone or with any additional associated plant. This is also the case for bare soils (Fig. 3).

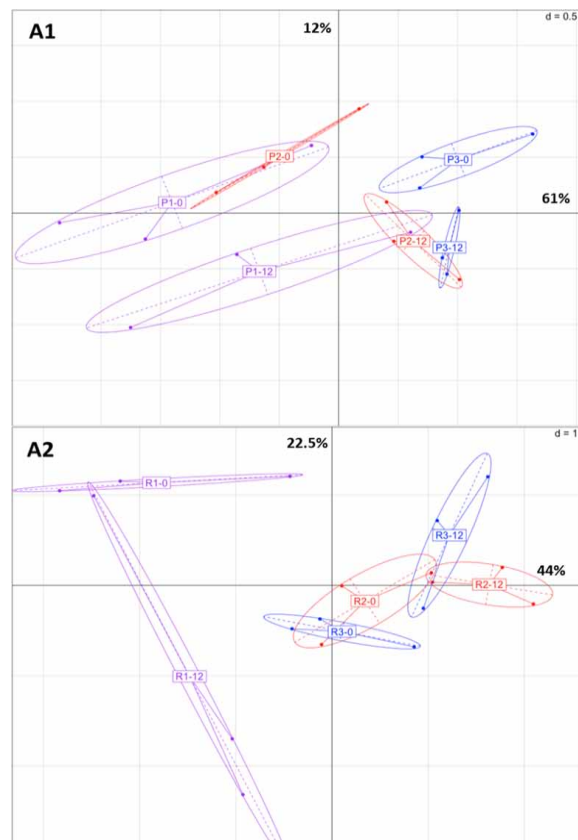


Fig.2. BCA analysis of soil bacterial community structure in the pristine forestland.

(A1) and recolonized Sand site (A2) including samples from bulk soils (purple ellipses), *R. monosperma* rhizospheric soils (red ellipses) and *L. creticus* rhizospheric soils (Blue ellipses). Note: 0 and 12 after sample name correspond to time sampling.

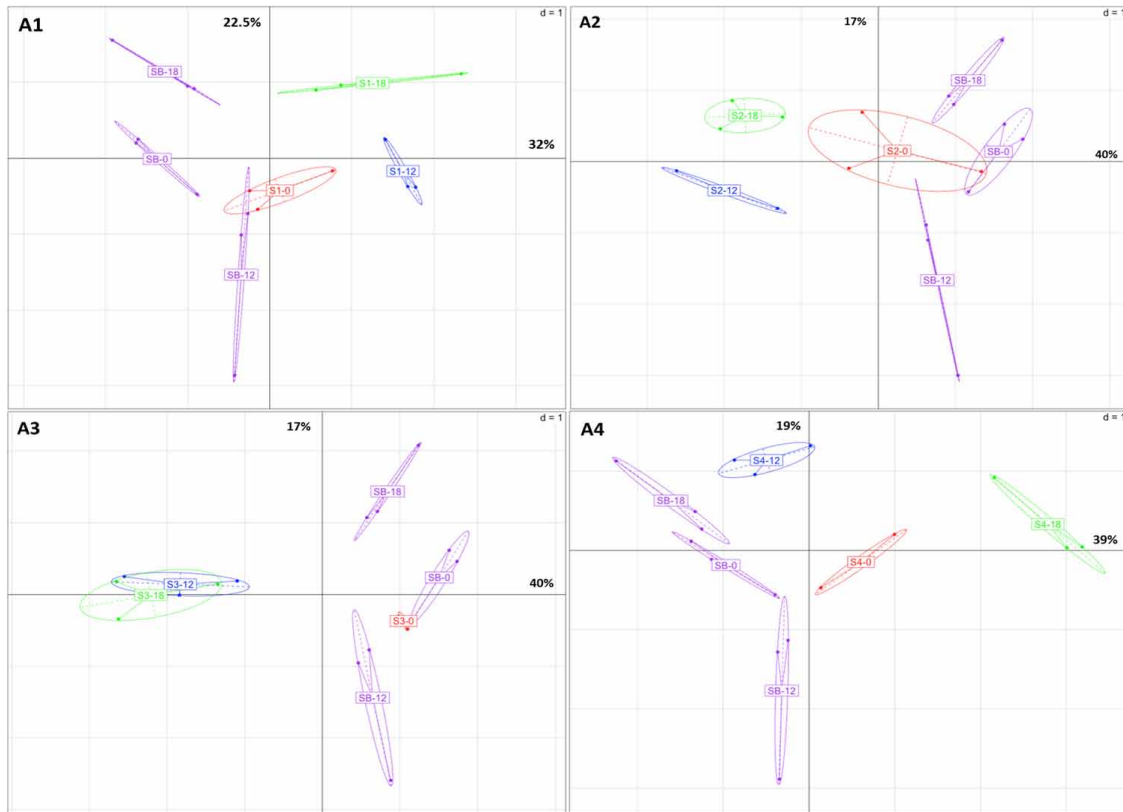


Fig. 3. BC Analysis of soil bacterial community structure of sand spoiltrial with *T. articulata*.

A1: Monospecific *T. articulata* rhizospheric soil samples; A2: Association of *T. articulata* and *R. monosperma* rhizospheric soils; A3: Association of *T. articulata* and *L. creticus* rhizospheric soils; A4: Association of *T. articulata*, *L. creticus* and *R. monosperma* rhizospheric soils. Samplings were performed at initial stage (red ellipses), 12 months (blue ellipses) and 18 months (green ellipses). Purple ellipses represent bulk soils whatever the sampling time.

At T0 the rhizospheric bacterial community structure is close to bare soil bacterial community structure. Considering *T. articulata* (S1) and its associations with *R. monosperma* (S2) or *L. creticus* (S3), BCA analyses support a temporal evolution as bacterial community structures of soil samples at T0, T12 and T18 (Fig. 3A1, 3A2, 3A3). The first axis explains 32.4; 37.2 and 39.8 % of the variance for S1, S2 and S3 respectively, demonstrating an evolution of bacterial community structure between bare soils and T12 soil samplings. Considering the last pattern (S4) representing the association of the three plants, the rhizospheric effect on bacterial community structure clearly occurred only at T18. The RISA profiles of bacterial communities of T18 soil samples are separated by the second axis, except for S3 patterns for which no evolution was observed between T12 and T18. Considering *S. terebinthifolius*, the rhizospheric bacterial community structures were stable and very

similar to those in bare soils (Fig. 4), whatever its associated plant(s) or time.

Relationships between bacterial community structure and physicochemical characteristics of soils

The results of a coinertia analysis (Fig. 5) indicate that the bacterial community structure and the physicochemical characteristics of soils are correlated ($P=0.036$; RV-coefficient=0.55). The first axis accounted for 84% of the explained inertia while the second axis accounted for 6 % of the explained inertia (Fig. 5). The first axis separated bulk soils and rhizospheric soils under *T. articulata* or *S. terebinthifolius* from the other plant combinations. For these soils, bacterial community structure and physicochemical characteristics are clearly correlated. In the other rhizospheric soil samples combining two or three plants, the correlation is less obvious.

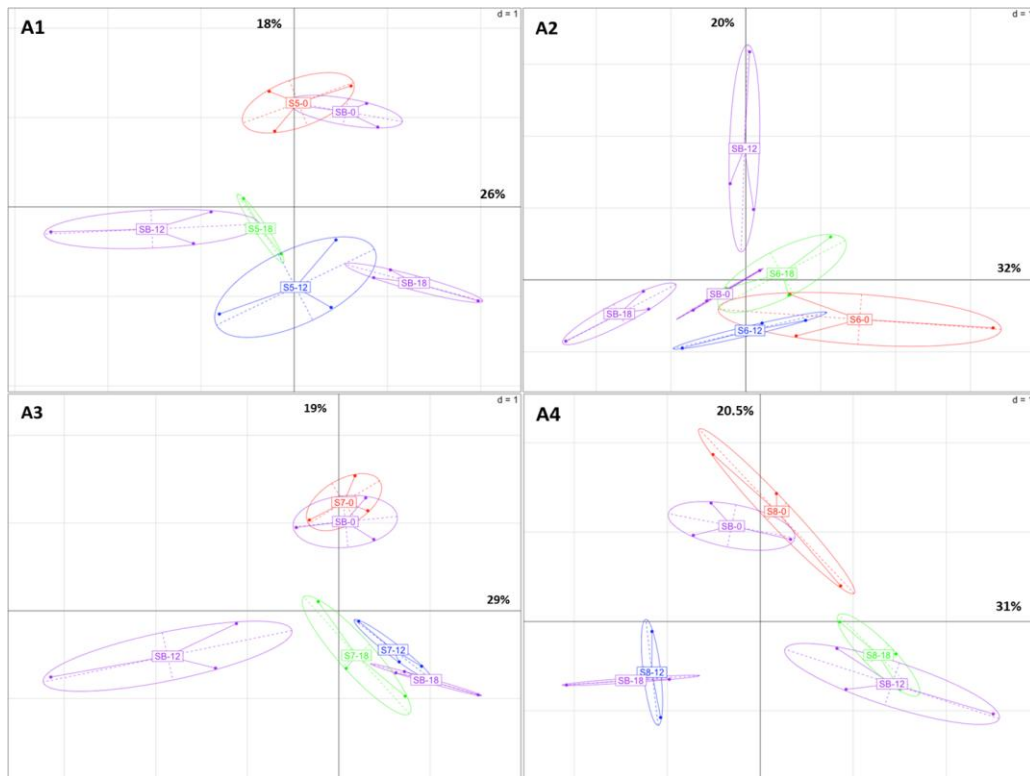


Fig. 4. BCA analysis of soil bacterial community structure of sand spoil revegetation trial with *S. terebinthifolius*. A1: Monospecific *S. terebinthifolius* rhizospheric soils; A2: Association of *S. terebinthifolius* and *R. monosperma* rhizospheric soils; A3: Association of *S. terebinthifolius* and *L. creticus* rhizospheric soils; A4: Association of *S. terebinthifolius*, *L. creticus* and *R. monosperma* rhizospheric soils. Samplings were performed at initial stage (red ellipses), 12 months (blue ellipses) and 18 months (green ellipses). Purple ellipses represent bulk soils whatever the sampling time.

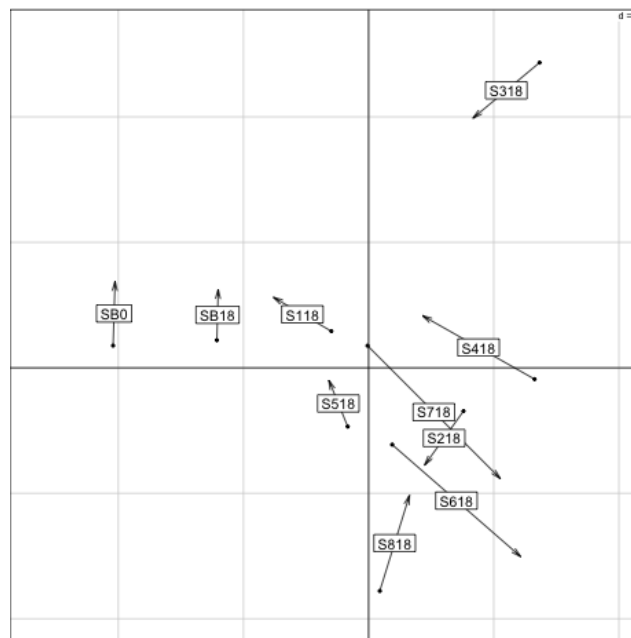


Fig. 5. Correlation between bacterial community structure and physicochemical characteristics by co-inertia analysis. Each site is represented by two points: one corresponds to the soil bacterial community structure and the other corresponds to the soil physicochemical characteristics. Note: 0, 12 and 18 after sample name correspond to time sampling.

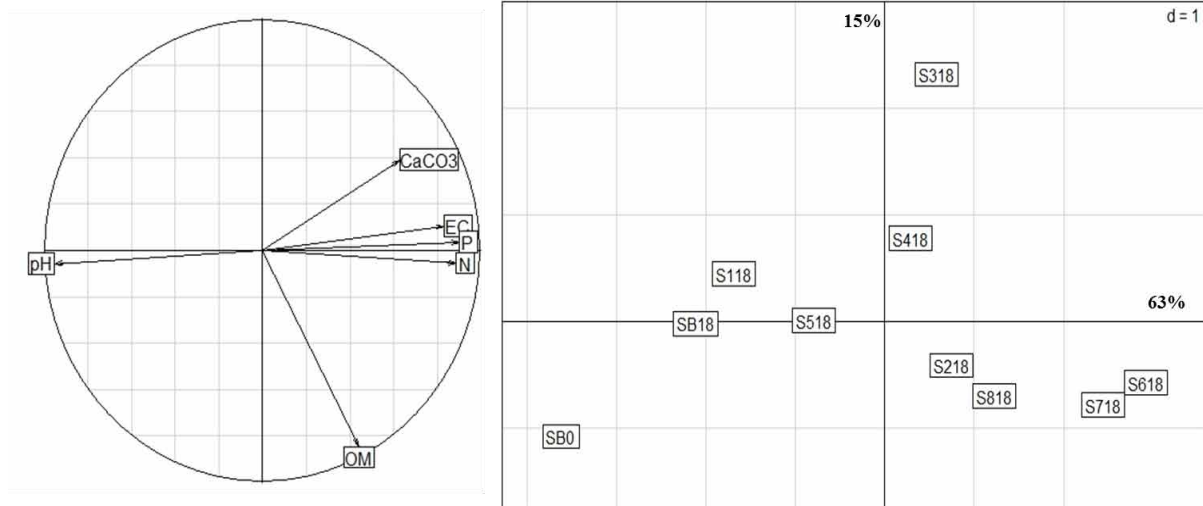


Fig. 6. S1. PCA analysis of soil physicochemical characteristics.

SB: Bulk soil; S1: Monospecific *T. articulata* rhizospheric soil samples; S2: Association of *T. articulata* and *R. monosperma* rhizospheric soil; S3: Association of *T. articulata* and *L. creticus* rhizospheric soils; S4: Association of *T. articulata*, *L. creticus* and *R. monosperma* rhizospheric soils; S5: Monospecific *S. terebinthifolius* rhizospheric soils; S6: Association of *S. terebinthifolius* and *R. monosperma* rhizospheric soils; S7: Association of *S. terebinthifolius* and *L. creticus* rhizospheric soils; S8: Association of *S. terebinthifolius*, *L. creticus* and *R. monosperma* rhizospheric soils.

Note: 0 and 18 after nomenclature represent time sampling.

Discussion

The extensive exploitation of Terga sandpit since 1941 (Ghodbani, 2008) induced a continuous and drastic decrease of vegetation cover. For tentative environmental balance of this site, several revegetation programs were achieved. In 1998, the Algerian Forest Service settled a recolonization trial plot by introducing two legume plant species, *Retamamonosperma* and *Acacia saligna*, unsuccessful for these species but with relative success of colonization by others plant species. Our research group settled additional trials in 2008, including legume plant-microorganisms symbioses. A successful sustainable restoration strategy requires many attempts during several years. Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (Harris, 2003). Indicators are necessary to evaluate the success or failure of revegetation attempts. Soil microbial community structure and evolution could be one of them to follow up soil quality and progress of revegetation (Sparling, 1992; Harris, 2003; Gomez *et al.*, 2006).

In the present study, the evolution of the bacterial community structure was registered, at spatial and temporal scales, in three cases: a recent revegetation trial (0-18 months after plantation), the 14 year-old recolonization trial site of the National Forest Service and the native undisturbed forest. In this purpose, we performed RISA, a molecular fingerprinting method widely reported in literature for successful monitoring of microbial communities in complex environments (Borneman and Triplett, 1997; Ranjard *et al.*, 2000a,b; Yu and Mohn, 2001; Eriksson *et al.*, 2003; Ikeda *et al.*, 2008).

Plant impacts on soil bacterial community structure and temporal evolution are not similar in the two older sites. Considering the native forest, bacterial community structures of both bulk and rhizospheric soils are close, whatever the plant species. This might be explained by the difficulty to find bare soil free of roots in this ecosystem at sampling. This suggests that natural processes may drive a dense vegetation cover in this dune environment. Opposite, in soils of the 14 year-old recolonized site, the bacterial communities are impacted by the plant rhizosphere. Soils of this site appear in a transitory state fourteen years after plantation.

This could be explained by the fact that plants introduced by national forest service did not persist but allowed colonization by other species. It is difficult to predict the period of time necessary for this ecosystem to come up to resilience because there are several processes and factors that influence ability and rate of recovery (Lal, 1997).

The soil bacterial community structure in these two sites is rather similar during temporal scale. This suggests stability over time, probably due to the lack of human disturbance. In fact, diversity of soil bacterial communities is impacted by natural (Paine and Levin, 1981; Sousa, 1984) or anthropogenic disturbances (Buckling *et al.*, 2000; Hery *et al.*, 2003; Kang and Mills, 2004; Herrera *et al.*, 2007). In order to understand impact of anthropic disturbance in Terga sandpit, the structure of bacterial communities has been followed in bulk and rhizospheric soils of the newly revegetated sandspoil. Major changes in soil bacterial community structure were observed in this recently revegetated sandpit, at the beginning of the planting and in some cases.

The introduction of *T. articulata* (Barbary Thuja) was unsuccessful as plants died after one month. Nevertheless, although *T. articulate* was planted alone, a plant rhizospheric effect on bacterial communities was observed after 12 months. To explain this, we may hypothesize that the soil and its associated bacterial community introduced with the plant may have had an impact on bacterial diversity. This suggests that, in case of future revegetation attempts on Terga sandpit, topsoil could be used to boost soil biology and plant growth. Topsoils have been used with success in others environments Warren *et al.*, 1980; Rokich *et al.*, 2001; Wong, 2003; Rate *et al.*, 2004; Mola *et al.*, 2009). Association of *T. articulata* with *R. monosperma* or *L. creticus* had an effect on soil bacterial community structure, especially with *L. creticus*. Nevertheless, association of the three plants together mitigates this impact. Plant diversity is a driver of bacterial community structure and may decrease or cancel impacts of individual plants by mediating microbial interactions like competition, antagonism (Schlatter *et al.*, 2015).

S. terebinthifolius (Brazilian pepper tree) characterized by a high growth rate and a wide environmental tolerance was introduced in more than 20 countries (Ewel *et al.*, 1982) to invade a variety of ecosystems (Woodall, 1982; Laroche and Baker, 1994; Langeland and Burks, 1998). The introduction of this plant in Terga site was a success as the plant grew well during the 18 month-period of this study. Nevertheless, a weak effect on bacterial community structure was observed, whatever *S. terebinthifolius* was alone or associated with legumes. This could be explained by allelopathic properties of this exotic invasive plant (Mahendra *et al.*, 1995; Nickerson and Flory, 2015), which may prevent the development of others plants and may influence the bacterial community structure.

Plants also impact concentration of soil nutriments (OM, C, N, P and K) that are drivers of competition and interactions between soil bacteria (Schlatter *et al.*, 2015). Moreover, soil edaphic parameters drive bacterial community structure. Many authors reported relationships between edaphic parameters and microbial community structures in soils (Bardgett *et al.*, 1997; Fierer and Jackson, 2006; Lejon *et al.*, 2007; Lauber *et al.*, 2008; Lauber *et al.*, 2009; Nacke *et al.*, 2011). In the Terga trial, plantation of legumes results in pH variations from alkaline to acidic, and induces increase of nitrogen and phosphorus contents. A correlation between soil parameters and bacterial community structure was evidenced by coinertia analysis. These findings suggest that plants drive bacterial community structure by producing root exudates and by modifying soil physicochemical parameters. Literature reports that root exudates create favorable environment for soil microorganisms, by secreting 10 to 20% of carbon assimilated during photosynthesis (Dakora and Phillips, 2002; Walker *et al.*, 2003; Hartmann *et al.*, 2009; Dennis *et al.*, 2010; Dazy *et al.*, 2008; Siciliano *et al.*, 2003).

In conclusion, although all plant introduction assays were not successful, we evidenced changes in soil edaphic and biotic characteristics. Moreover, bacterial community structure analyses suggest that the recolonized site may be in an intermediate state between the native forest and the revegetated site.

This raises the question of the time necessary to reach the ecological resilience in this ecosystem. This work provides new data that could be used for the restoration of Terga sandpit. Specifically, in future more care should be provided on plant selection and association and characterized topsoil may be spread on the trial before planting.

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