

## Bio-Revegetation Impact on the Physicochemical Characteristics of a Sandy Quarry Soil in Terga Beach Region in Algeria

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### Abstract

In order to define the impact of a bio-revegetation effect on soil physicochemical properties, we used *Acacia Saligna* in variants with bio-fertilizers such as rhizobia and mycorrhizae that play a key role in the productivity and sustainability of soil as well as the environmental protection. The area of study is a degraded sandy quarry in Terga, a coastal semi-arid area located in the northwestern part of Algerian. Our sampling and analysis of soil were made after each trimester of experiments in the fields, using four blocks, each one containing ten plots. Sampling is a composite of soil that was made in each plot diagonally on a depth of 10 cm and a diameter of 30 cm from the plant, at different times: first trimester (3 months), second (6 months), and third (9 months). Preliminary results showed a real and favorable modification of substrates by obtaining materials with less alkaline pH, there is a significant phosphorus increase in the second and third trimester compared to the first trimester, however the soil calcareous nature prevents the expression of some parameters resulting in a small improvement in total nitrogen and a deficiency in both exchangeable magnesium and organic matter.

**Keywords:** sandy quarry, revegetation, *Acacia Saligna*, rhizobia and mycorrhizal inocula, total nitrogen, available phosphorus

### Abbreviations

Myc: Single endomycorrhizae inoculation. Con: control.

S10, S14, S24: Single Rhizobial inoculation.

D10, D14, D24: Dual Rhizobial + Endomycorrhizae inoculation.

Mix: mixed Inoculation with the 3 rhizobial strains (S10+S14+S24) + endomycorrhizae.

CT: Total limestone, CA: active limestone, Ca: exchangeable calcium, Mg: exchangeable magnesium.

P: available phosphorus, N: total nitrogen.

T1, T2, T3: first trimester, second trimester, third trimester.

### 1. Introduction

The soil is the living epidermis of earth, at the interface between the atmosphere, rocks and the living world. It is the meeting point of the plant world, animal and mineral that provides primary production on which human population, flora and fauna depend directly. Soil participates in the great cycles of energy, water and elements (Robert, 1996). It is essential to human activities and the functioning of terrestrial ecosystems. The soil is no longer considered an inert medium. It evolves in space and time. This development gives it variability in its morphological, physical, chemical and biological properties (Collin, 2006). However; it stays a nonrenewable resource because of the long time required for its formation process. Therefore, its preservation and restoration by biological and non-aggressive means to the environment is a major challenge of sustainable development.

In order to re-vegetate a degraded sandy quarry located in Terga (Province of Ain Témouchent in northwest Algeria), *Acacia saligna* plants with rhizobia (nitrogen fixing symbiont) and mycorrhizal inocula were used.

Originally from Australia *Acacia saligna* is a nitrogen-fixing legume, introduced in Algeria, which is characterized by rapid growth and tolerance to drought, salinity and alkalinity; despite the fact that it has a short life varying between 10 and 20 years (Maslin & McDonald, 2004). However, microbial inocula or bio-fertilizers that have been used on an experimental basis in the soil can be defined as a preparation containing live and efficient microbial cells whose role is to increase the number of these microorganisms, and accelerate microbial processes, thus increasing the assimilation of some plant nutrients such as nitrogen and phosphorus. The goal of our work is to investigate the impact of this bio-revegetation on the soil edaphic aspects over time.

## 2. Materials and Methods

### 2.1 Study Area

The experimental study site, chosen according to its highly degraded character of quarry pit-end operating land, was located in the town of Terga, which extended over an area of 65.07 km<sup>2</sup> (average central location at latitude 35°25'07" North and Longitude 1°10'39" West). It covered an area of 120ha located at the mouth of the Oued El Malah, about 7 km from Terga village (Province of Ain Témouchent). The average altitude of this vast terrace, gently sloping towards the sea, was between 200 and 400 m (Figure 1).

#### a. Detailed view :

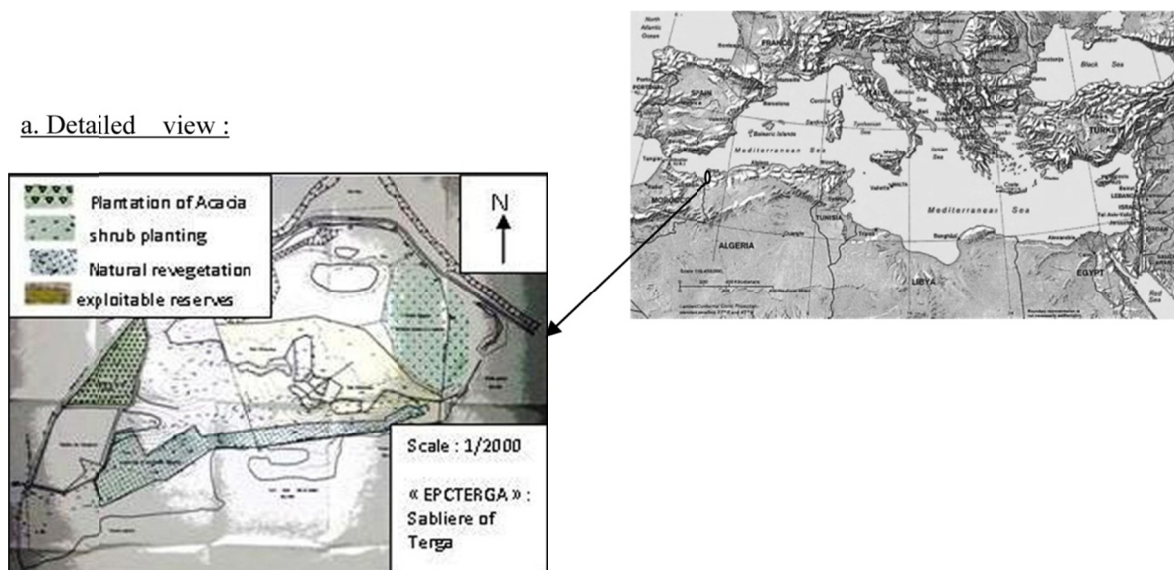


Figure 1. Map location of the study area (scale: 1/19.450.000; detail:1/2000)

### 2.2 Re-Vegetation Site

The experimental site of the old quarry revegetation contained four blocks, each containing ten plots: A non *Acacia Saligna* plot (bare soil), an *Acacia Saligna* plot (control), three plots of *Acacia saligna* with single rhizobial inoculation with three selected strains: S10, S14 and S24 based on their efficiency *in vitro*, a plot of *Acacia saligna* with single mycorrhizae inoculation (Myc), three plots of *Acacia Saligna* with dual inoculation rhizobial strain S10, S14, S24 + mycorrhizae (D10, D14, D24), and finally a plot of *Acacia Saligna* with mixed inoculation S10+S14+S24+ mycorrhizae (Mix). The layout of all plots and blocks is shown Figure 2.

### 2.3 Materials

#### 2.3.1 The Rhizobial Strains

The bacterial strains were isolated from nodules of *Acacia Saligna* of 6 plants *in natura* from Terga area. Each nodule was washed successively with water and then disinfected in sodium hypochlorite 12% for 1 min and then rinsed 10 times with sterile distilled water. Nodules were crushed under aseptic conditions on YMA medium. However, purification of colonies was obtained after a study of their phenotypic characteristics. Afterward, the best and efficient strains were got via nodulation test and abiotic factors test (Temperature, pH, salinity) (Mansouri, 2011).

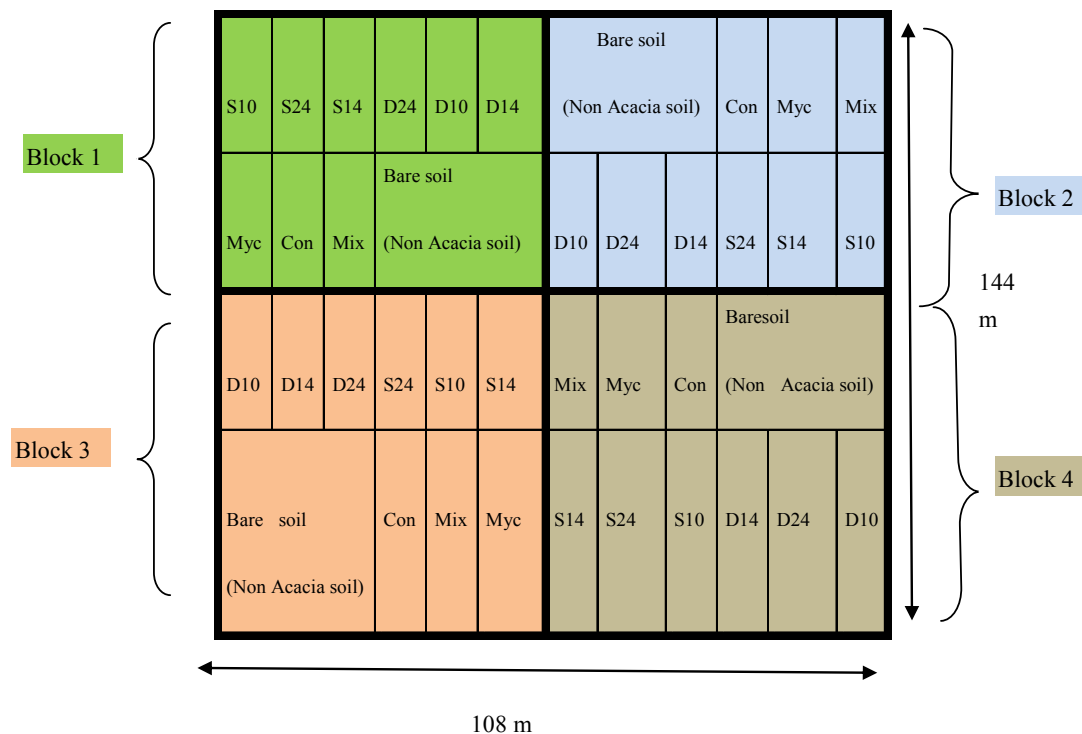


Figure 2. Presentation of the device to test plots distributed in complete blocks in total randomization

### 2.3.2 Inoculum Preparation

An isolated colony was inoculated in an Erlenmeyer flask of 500 ml containing 100 ml of (YMA) broth which was already sterilized at 120° C for 20 min (Vincent, 1970). After 5-7 days of incubation, 100 ml of each inoculum containing about  $30 \times 10^8$  bacteria/ml was transferred into a 2 L erlenmeyer flask filled up to 1 L to allow aeration of medium during agitation (Mansouri, 2011).

### 2.3.3 Test Nursery Inoculation

*Acacia Saligna* seeds were already scarified with  $H_2SO_4$  for 90 min, after they were rinsed with sterile distilled water and then germinated in water agar 0.8% (Tillard & Drevon, 1988). After 6 days, seeds were transplanted into plastic bags and then transferred to nursery. The soil used was composed of 25% of the career's sand and 75% of peat (Mansouri, 2011). Each 5 ml of a pure culture of rhizobia containing approximately  $30 \times 10^8$  bacteria/ml was used in the soil at the collar when transplanting. One week after the seedlings, plantlets received a second inoculation (Diouf et al., 2003; Mansouri, 2011).

### 2.3.4 Mycorrhizal Inoculums Preparation

*Acacia Salina* fresh roots were collected from 7 trees of *Acacia Salina* in Terga town and 7 other trees in the city of Oran. At each site (Terga and Oran) samples of fine roots were removed from *Acacia Saligna* root system, and then they were well washed, then submerged in a 20% KOH solution for 20 min at 90°C. They were then thoroughly rinsed with water and soaked in a 1% HCl solution for 5 minutes. Roots were then stained in a solution of 0.1% trypan blue in lactophenol for 20 minutes (Philipps & Hayman, 1970). About 50 random fragments of roots thus treated were cut to pieces of about 1cm length and compacted between slides and layers. Fragments were then observed under a light microscope 10x40 to estimate the endomycorrhizal frequency (Mansouri, 2011).

### 2.3.5 Nursery Inoculation Tests

Roots mycorrhization whose frequency rate was 100% were used, an application of about 1g of fresh endomycorrhizal roots against each of the root system at the time of transplanting plantlets (Mansouri, 2011). After 8 months of warehousing in the nursery, the plantlets were transferred to the field (Mansouri, 2011).

### 2.4 Sampling

Sampling is a composite of soil (from reworked materials) that was made in each plot diagonally on a depth of 10 cm and a diameter of 30 cm from the plant at different times T1 (3 months), T2 (6 months), and T3 (9 months)

(Figure 3). The soil is dried in the open air for a week and then crushed and sieved through a diameter of 2mm to obtain the fraction of fine soil to perform a set of physico-chemical analysis.



a. Seedlings of *Acacia Saligna*, the planting day(T0)      c. *Acacia Saligna*, 6 months after planting (T2)



b. *Acacia Saligna*, 3 months after planting (T1)      d. *Acacia Saligna*, 9 months after planting (T3)

Figure 3. Re-vegetation sites at different trimester after plantation T0, T1, T2, T3

## 2.5 Physical Analysis

### 2.5.1 pH Measurement

About 20 g of soil was weighed, 50 ml of boiled distilled water was added to it and the mixture was stirred vigorously for 2 hours. The content of the beaker was abandoned then the pH is measured using a pH meter after a brief agitation (Aubert, 1978).

## 2.6 Chemical Analysis

### 2.6.1 Identification of Organic Matter by Calcination

The soil sample was dried overnight (16 hours) at 150°C. The crucible was cleaned by heating to red then was cooled in a desiccator for 10 minutes. The empty crucible was weighed, then 10 g of dried soil was added and the final weight was recorded. Afterward, the soil was calcined in a muffle furnace at 375°C for 16 hours. Finally the crucible containing the ashes was cooled in a desiccator and weighed (Centre d'expertise en analyse environnementale du Québec, 2003).

### 2.6.2 Measurement of Total Limestone Content by the Bernard Calcimeter

1 g of soil was weighed, and then 5 ml of HCl $\frac{1}{2}$  was introduced into the Bernard calcimeter finger. Next, the soil amount was weighed, and entered into the calcimeter vial after moistening it a little. The vial was closed by connecting to calcimeter, while the liquid of the graduated scale had to beat zero. The flax was then stirred to allow the mixture of the soil with HCl. This agitation resulted in the release of CO $_2$ . When the liquid level was stable the CO $_2$  volume was read. The calcimeter was calibrated with pure anhydrous CaCO $_3$  (Aubert, 1978).

### 2.6.3 Measurement of Active Limestone Content by Drouineau – Galet Method

250 ml of ammonium oxalate 0.2 N was added to 10 g of soil, the content was stirred for 2 hours and was filtered. In a 100 ml beaker, 10 ml of the filtrate was poured as well as 10 ml H $_2$ SO $_4$  1/10. The beaker was placed in an oven at a temperature of 60°C, afterward it was placed on a magnetic stirrer surmounted by a buret containing potassium permanganate solution N/10. The permanganate was titrated until we obtained a persistent pink color. n was the

number of milliliters of  $\text{KMnO}_4$  poured. In the same way 10 ml of solution of ammonium oxalate was titrated, N was the number of milliliters of  $\text{KMnO}_4$  poured for control (Aubert, 1978).

#### 2.6.4 Measurement of Total Nitrogen Content by the Kjeldahl Method

1 g of the soil was weighed and was put into a digestion flask containing 12-15 ml of concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ ), then 7g of potassium sulfate and a catalyst as Copper were added, the digestion was brought to a "rolling boil" ( $370^\circ\text{C}$  to  $400^\circ\text{C}$ ) and the mixture was heated until white fumes could be seen; then 250 ml of water was added. The pH mixture was increased, with 45% NaOH solution, so the ammonium ( $\text{NH}_4^+$ ) ions were converted to ammonia ( $\text{NH}_3$ ), which was a gas that was distilled and then trapped in a special solution of about 15 ml HCl in 70 ml of water. Afterward, an indicator color was added to the trapping solution showing an important amount of trapped acid was still present. Afterward, a standard solution of NaOH was put into the buret and the trapping acid solution was titrated with the sodium hydroxide solution (Blamir, 2003).

#### 2.6.5 Measurement of Absorbed Phosphorus by the Olsen Method

1 g of soil was put into a 50 ml erlenmeyer flask, then 20 ml of extracting solution was added to each flask which was shaken at 200 rpm or more for 30 minutes at a room temperature, afterward extracts were filtered through Whatmann filter paper # 42, then phosphorus was analyzed by colorimetry or inductively coupled plasma emission spectroscopy using a blank and standards prepared in the Olsen P extracting solution (Hodges, 2000).

#### 2.6.6 Identification of Exchangeable Cations ( $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ ) by Atomic Absorption Spectrophotometry

10 g of soil was poured into a tube percolation mixing soil with a defined amount of quartz sand, after the bulb tube surmounting the percolation was filled of 500 ml ammonium acetate (77.08 g/l). The percolation took place during about 8 hours. Then percolate was collected into a 500 ml flask. Finally, the percolating was up to 500 ml with a solution of ammonium acetate (Aubert, 1978).

The Measurement of calcium rates was done by establishing a calibration range of 0, 4, 8, 12, 16 and 20 ppm from  $\text{Ca}^{+2}$  (40 ppm) solutions. The extract solution was diluted to obtain a concentration of less than 20 ppm (Aubert, 1978). The measurement of magnesium rates was done by establishing a calibration range of 1, 2, 3, 4 and 5 ppm from magnesium solutions (20 ppm) each complete 100 ml flask, with distilled water (Aubert, 1978).

### 2.7 Statistics Analysis

The average concentrations of various parameters analyzed are affected by an analysis of variance using the Fisher's exact test at  $P=5\%$  using the software SPSS 8.0 for windows.

## 3. Results and Discussion

We note that the non re-vegetated plots recorded an increased pH values over time: the reported T1 (summer) pH is lower than that of autumn (T2) which is lower than that of winter (T3) (Figure 4.a). There is a significant increase of pH ( $p<0.05$ ) in T2 compared to T1 on control plots (8.37 compared to 8.04), S14 (8.46 compared to 7.99), D10 (8.52 compared to 8.06), D14 (8.43 compared to 8.04), D24 (8.65 compared to 7.97). There is also a significant increase of pH ( $p<0.05$ ) in T2 compared to T3 on the plots: D10 (8.52 compared to 8.05), D14 (8.43 compared to 8.09) and D24 (8.65 compared to 8.13). Moreover, there is a significant pH decrease at  $p<0.05$  in T3 on the plots of D10 and D14 relative to the non Acacia plots (8.05, 8.09 compared to 8.29). In fact, these results are closely related with the seasonal variations in pH, since pH rates decrease in hot dry weather and increase in rainy and cold weather (Gasser, 2011). However, the decrease in pH levels in T3 under a vegetative cover may be due to a release of protons ( $\text{H}^+$ ) during the uptake of cations by roots (Bye, 1999). In addition, the root exudates may indirectly lead to a decrease in pH because it stimulates the proliferation of soil microorganisms that synthesize organic acids and thus acidifies the soil (Davet, 1996; Waligora, 2010). The activity of soil microorganisms also depends on the temperature, moisture and soil texture (Lundquist et al., 1999; Steenwerth et al., 2008). Nevertheless, it was observed in laboratory conditions that rhizobia decreases soil pH; while Bradyrhizobia trends to increase it (Dubey, 2011). It was found however that the pH is a significant factor that affects the nodulation in soils mines with a high rate of nodulation at a pH which is between 5.5 and 7.2 and a low rate to a pH below 5.5 (Zahrn, 1999). Meanwhile, alkaline soils with a pH greater than or equal to 7.8 limit accessibility to Iron, Zinc, Manganese and mainly Boron and Phosphorus in soil, thereby reducing the Nitrogen fixation (Graham & Allan, 2002)

We found, a deficiency of exchangeable Magnesium for every plot at various times (T1, T2, T3) (Figure 4.b), with rates well below the standards that vary between 10% and 20% (Reuter et al., 1997). In fact, an excess of Calcium causes a Magnesium deficiency. However, an excess of Potassium inhibits the absorption of magnesium (Pousset, 2002). Erosion could also be one of the main causes of magnesium deficiency (Boyer, 1978). Thus, the soil is moderately calcareous (Figure 4.c), however, there is a significant decrease at  $p<0.05$  in the total content of

limestone at T2 in S14 plots (15.32%) compared to bare soil (18.55%) and a significant increase ( $P < 0.05$ ) at T3 in D24 plots (19.22%) compared to non Acacia plots (18.45%). Similarly, we recorded a significant increase ( $p < 0.05$ ) at T2 and T3 compared to T1 in Myc plots (18, 12, 12% and 19% compared to 12.83%) and mix (18.9% and 18.77% compared to 13.15%) and a significant increase ( $p < 0.05$ ) at T3 compared to T1 in S14 plots (19.1% compared to 14.53%).

This limestone provides divalent calcium  $\text{Ca}^{2+}$  that is present especially in the colloid as an exchangeable cation which is the form used by plants (Khan Towhid, 2013). The Calcium content at T2 increases significantly ( $p < 0.05$ ) in D24 plots compared to control and non Acacias plots (88.98 meq/100g compared to 50.1 meq/100 g and 50.23 meq/100g). There is also a significant increase ( $p < 0.05$ ) in T2 compared to T1 in S14 plots (63.63 meq/100g compared to 17.93 meq/100g), D24 (88.98 meq/100g compared to 24.75 meq/100g); and mix plots (50.98 meq/100g compared to 20.03 meq/100g) and a significant increase ( $p < 0.05$ ) in T2 compared to T3 in D24 plots (88.98 meq/100g compared to 27.16 meq/100g) (Figure 4.d).

The level of active limestone changes but not significantly. However its content is close to or exceeds 5% (Figure 4.e). Therefore, limestone solubilization and the gradual release of Calcium can be achieved by acid rainwater or by the biochemical activities of either microorganisms or plant roots (Wierzchos et al., 2003; Salomon, 2006; Coque, 2008). Moreover, the abundance of Calcium ions has an antagonistic effect on other nutrients availability such as Potassium, Iron, Boron, Copper, Manganese, Zinc and signs of deficiency or fading can appear on plants (chlorosis) (Pousset, 2002; Vasant et al., 2009; Ofme, 2011; Morel, 1996). In addition, it inhibits the mineralization of organic matter under the effect of coating (Morel, 1996). Although, land may be rich in total limestone, but relatively poor in active limestone (Pousset, 2002).

The Phosphorus level is low between 1 and 9 ppm. (According to AgSource Laboratories, 2013) (Figure 4.f). However there is an evolution in content over time, where there is a significant increase ( $p < 0.05$ ) of phosphorus in T2 compared to T1 in S10 (6.78 ppm compared to 5.2 ppm), S14 (7.59 ppm compared to 5.82 ppm), S24 (7.16 ppm compared to 3.95 ppm), Myc (7.55 ppm compared to 4.67 ppm) and D24 (7.92 ppm compared to 5.32 ppm) plots. On the other hand, there is a significant increase ( $p < 0.05$ ) in T3 compared to T1 in D10 (7.45 ppm compared to 5.07 ppm) and Mix (7.77 ppm compared to 5.8 ppm) plots. In fact, Phosphorus is generally low in calcareous alkaline soils. It tends to be insolubilized by the Calcium (Calcium Phosphate and Magnesium) and it is possible that the phosphoric anions precipitate at the contact of the active limestone (Pousset, 2002; Ryan et al., 2001; Baize, 2000). However, Phosphorus is found in organic and inorganic forms. Low Phosphorus availability is due to the larger action of phosphoric anions soluble with  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ , depending on the geochemical soil properties (Gyaneshwar et al., 2002). Some microorganisms are able to solubilize phosphate minerals while reducing the pH by the secretion of organic acids which are good chelator of divalent cations such as  $\text{Ca}^{2+}$  and they can also form a complex with the metal ions associated with Phosphorus, thus releasing Phosphorus (Jones, 1998; Gyaneshwar et al., 2002; Pradipta, 2008). The mechanism of solubilization of inorganic Phosphorus is provided by organic acids; although the acid phosphatases play a major role in the mineralization of organic phosphorus (Goldstein, 1995; Kim et al., 1997; Rodriguez & Fraga, 1999). The genera *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful bacteria in the process of dissolution of Phosphorus (Rodriguez & Fraga, 1999). However, mycorrhizal hyphae make the Phosphorus and certain mineral traces such as Calcium and Zinc available to the host plant (Olsson et al., 1999). In agroforestry, mycorrhizal association contributes to the growth of Acacia species in infertile soils (Dart et al., 1991). Moreover, the dual inoculation by arbuscular mycorrhizal and bacteria that solubilize phosphorus increases the absorption of native soil Phosphorus as well as Phosphorus coming from Phosphate rocks (Goenadi et al., 2000; Cabello et al., 2005).

Total Nitrogen contents have not changed much except for the plots S14 where there has been a significant increase ( $p < 0.05$ ) in T3 compared to T1 (0.052% compared to 0.023%) (Figure 4.g). It is true that the bioavailability of Nitrogen depends primarily on the activity of nitrifying bacteria, but their activity is pH-dependent: they are, in fact, more active when the pH is between 6.5 and 7.5 and the optimum temperature between 30° to 35° Celsius (Biswas & Mukherjee, 2006). Although, microbiological antagonism may reduce nodulation (Kumara et al., 1974).

Organic matter levels determined are very low (<2%) (Figure 4.h). This result is in accord with Godwin (1992), who reported that soils in semi-arid regions contain little organic matter, of no more than one percent. Additionally, sandy soils are low in organic matter which generally would make them weak and unproductive (Pousset, 2002). In fact, minerals, organic matter and microorganisms are the key elements in pedogenesis and soil conservation (Bollag & Leyval, 1998).

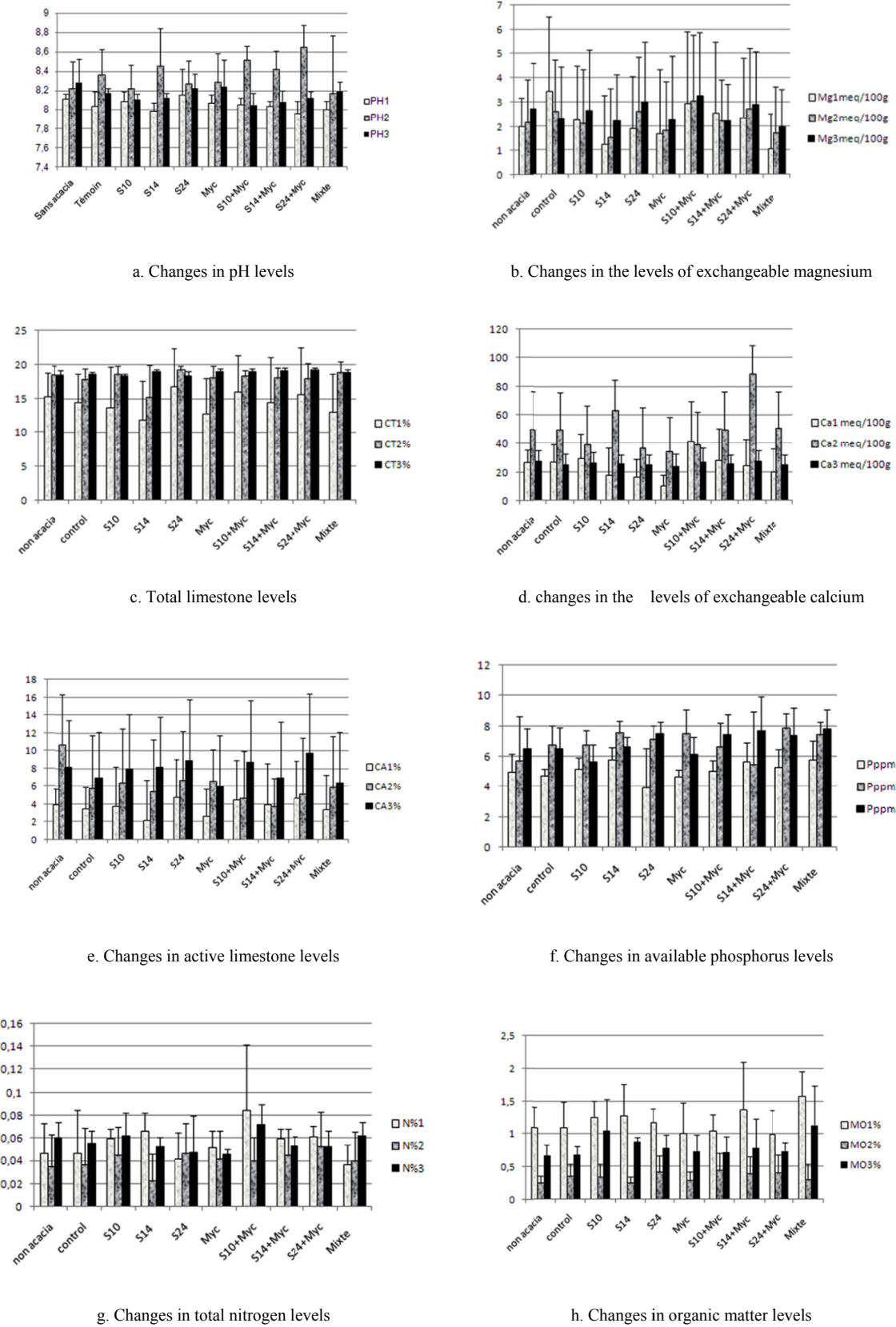


Figure 4. Changes in the levels of pH, exchangeable magnesium, total limestone, exchangeable calcium, active limestone, available phosphorus, total nitrogen and organic matter during the three trimesters T1, T2, T3

### 3. Conclusion

After nine months of a bio-re-vegetation, we could achieve at a less alkaline pH soil and an improvement in phosphorus content. However, the soil calcareous nature and the rate of either active limestone or the exchangeable Calcium results in a small increase in total Nitrogen and organic matter, and makes exchangeable magnesium contents deficient. In perspective, the factor time may be important in this study, so the experimentation can be conducted over a period of two years or more.

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