Use of Organic and Biological Fertilizers as Strategies to Improve Crop Biomass, Yields and Physicochemical Parameters of Soil

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#### Abstract

Interest in the sustainability of soil resources has been stimulated by increasing concerns that soil is one of the most critical components of the earth's biosphere,

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participating in food production and management maintenance of environmental quality. In arid and semiarid regions, oases appear to be a major threat model in the soil component. The palm plantations contribute to the formation of oasis ecosystems by promoting the creation of a suitable microclimate for the development of underlying crops and offsetting the effects of drought. These ecosystems play key roles in multiple socioeconomic and environmental issues. Nevertheless, they remain fragile and undergo impacts of human and/or natural origins permanently such as extreme temperatures, soil salinity, drought, erosion, and low contents in organic matter and native fertility. In order to ensure good yields, farmers use an intensive amount of chemical fertilizer, but it can have detrimental effects on soil. In this chapter, we will focus on the improvement of the biomass and yield of different agricultural crops – i.e., cereals (wheat, corn), vegetable crops (lettuce, tomato, leek), leguminous (alfalfa), and trees (date palm) – in field via the enrichment of soil by setting up an efficient biological protocol integrating arbuscular mycorrhizal fungi (AMF), PGPR, and/or organic soil conditioners resulting from green waste, phosphogypsum, phosphate wash sludge, and agroindustrial poultry waste manure. Our results confirmed the advantages of various biological and organic fertilizers in improving the biomass and yields for different crops. The combination of AMF and compost green waste appeared to be interesting for the improvement of the growth, mineral nutrition, and physiological and water parameters of date palm (*Phoenix dactylifera* L.). Furthermore, the combination of low dose (5%, 10%) and indigenous AMF is clearly beneficial for the growth of alfalfa and tomato under a greenhouse. Concerning the experiments carried out in the field, it confirmed the advantages of biological and organic fertilizers in improving the yield for leguminous (alfalfa), vegetable crops (lettuce, tomato, and leek), and cereals (wheat). Application of the tripartite combination AMF-PGPR compost was more efficient in increasing the yield of the tested plants. Indeed, biological treatments had an important effect on the physicochemical properties of the soil. Finally, we have elucidated the positive impacts of biofertilizers used and the interest of adopting the innovative practices improving soil fertility, preserving water resources, respecting the environment, and ensuring the development of sustainable organic agriculture.

#### Keywords

Climate change  $\cdot$  Compost  $\cdot$  Arbuscular mycorrhizal fungi  $\cdot$  PGPR  $\cdot$  Symbiosis  $\cdot$  Soil degradation  $\cdot$  Date palm  $\cdot$  Yield  $\cdot$  Soil management  $\cdot$  Sustainable agriculture  $\cdot$  Underlying crops

#### **Abbreviations**

AMF Arbuscular mycorrhizal fungi F Mycorrhizal frequency Foa Fusarium oxysporum f. sp. albedinis

MPN Most probable number

PGPR Plant growth-promoting rhizobacteria

GWDL Grass waste and dead leaves

GWSP Grass waste and sludge of phosphate

OCOMWWG Olive cake, olive oil mill wastewater, and garbage

PGGW Phosphogypsum and green waste PMGW Poultry manure and green waste

R Stomatal resistance
RWC Relative water content
SOC Soil organic carbon
SOM Soil organic matter
TOC Total organic carbon
TKN Total Kjeldahl nitrogen

#### 9.1 Introduction

Interest in the sustainability of soil resources has been stimulated by increasing concerns that soil is one of the most critical components of the earth's biosphere, involved in producing food and maintaining different scales of environmental quality. At the same time, the function of supporting world food and agriculture is a key for the preservation and advancement of all life on planet Earth. However, the decline in the soil quality around the world is a challenging task and will likely remain an important global issue in the years to come. International attention to protecting the environment has been increasing in recent decades focusing on protection from global warmth and desertification, which has affected the ecosystems worldwide. In arid and semiarid regions, oases appear to be a major threat model in the soil component. Therefore, it is important to understand the spatial distribution characteristics of the soil and the management practices to provide a wide range of ecosystem services and specific sustainability benefits associated with improving soil health practices. Oasis environment is considered as a model ecosystem where agriculture is possible by the microclimate determined by the date palm (*Phoenix* dactylifera L.) for the development of underlying crops (arboriculture, cereals, and horticultural crops) and offsetting the effects of environmental stresses (Ehsine et al. 2014). This ecosystem plays key roles in integrating economic, social, and environmental issues. Nevertheless, this agroecosystem remains fragile and often vulnerable to human and/or natural impacts such as urbanization, abiotic (i.e., heat, drought, salinity, desertification, depletion of soil organic matter, and nutrients) and biotic stresses (i.e., bayoud palm caused by Fusarium oxysporum f. sp. albedinis (Foa)), genetic erosion, and aging (Oihabi 1991; Saaidi 1992; Ziouti 1998; Botes and Zaid 2002; Awad 2006; Jaiti et al. 2008; Meddich et al. 2015a; Meddich and Boumezzough 2017; Ashoka et al. 2017; Kumar et al. 2017b).

In the oasis, the date palm is the oldest and most widely cultivated tree that is commercially the most important tree in the life of its people and their heritage. The importance of the date palm occurs because of its great contribution to the creation. maintenance, and development of the economy in the oases. The economic utility of these palms is multifold, including staple food, beverages, ornamentals, building wood, and industrial materials (Balick and Beck 1990; Meena et al. 2015d; Gogoi et al. 2018). In addition to its commercial and nutritional value, the date palm tree has a minimum water demand, tolerates harsh weather, and tolerates high levels of salinity. Morocco, one of the important countries for date palm cultivation, has 41% of the world's date palm trees (14% of the date production), and nearly 340 of 2000 varieties recorded around the world are grown here. Morocco occupied the 3rd largest producer countries (Oihabi 1991) with  $15 \times 10^6$  date palm trees at the end of the nineteenth century. However, this number currently decreased dramatically to  $6.6 \times 10^6$  palm trees spread on 51,000 ha (FAOSTAT 2018). Yet it's important to note that this shrinking owing to bayoud disease is caused by Foa, tree aging, lack of maintenance, and the environmental conditions affecting negatively the development of date palm and its underlying cultures (FAO 2012). In addition, the low contents in organic matter and native fertility remain one of the major constraints to date palm production. As a result of these circumstances and to ensure good yields, many farmers increase the frequency of watering and the use of an intensive amount of chemical fertilizer to increase the level of nutrients found in soil. Inorganic fertilizers, which are highly absorbed by the ground, enhance plants' growth and yields of fruits and vegetables in a relatively short period of time leaving the rest of the chemicals to leach. As a result of leaching, the chemical fertilizer adversely affects soil chemical properties, water irrigation, and the amount, activity, and diversity of microorganisms beneficial to plant and soil health. The need to respond to these situations by adopting appropriate and sustainable strategies to ensure the protection and restoration of our oasis is a time-demanding task. Organic and biologic fertilizers such as arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and compost have emerged as safe and effective alternatives to the chemical fertilizers in order to improve the sustainability of agroecosystems as well as to increase soil quality and crop production per unit area of arable land.

AMF are a key integral component of the soil rhizosphere and are essential for the stability, sustainability, and functioning of the ecosystems. The external mycelium of AMF, considered as an extension of host plant roots, acts as a direct link between roots and soil nutrient reserves. The abundance of extraradical hyphae is a major factor in soil structure as they promote soil aggregate formation, which is important in resistance of soil erosion. The roots of date palm are receptive to the AMF and are capable to grow in the arid area (Oihabi 1991; Al-Yahya'ei et al. 2011; Meddich et al. 2015b; Meena and Meena 2017; Yadav et al. 2017c). The positive effects of mycorrhizal symbiosis on the growth and health of date palm have been reported (Al-Karaki 2013; Meddich et al. 2015a; Buragohain et al. 2017). Previous reports have revealed that AMF (1) promoted the growth of date palm seedlings in nursery conditions (Shabbir et al. 2011) than the controls treated with chemical fertilizers (Symanczik et al. 2014), (2) increased nutrient availability in soil cultures

(Al-Karaki et al. 2007), and (3) improved the absorption of water and nutrients under salt and drought stress conditions (Bearden and Petersen 2000; Baslam et al. 2013). Additionally, AMF were involved to improve the salt and drought tolerance in other crops such as lettuce (Ruiz-lozano et al. 1995; Baslam and Goicoechea 2012; Vicente-Sánchez et al. 2014), sorghum (Augé et al. 1995), corn (Subramanian and Charest 1997), clover (Oihabi and Meddich 1996; Meddich et al. 2000), and barley (Meddich 2001; Tao et al. 2014). AMF can protect also their host plant against biotic stress factors such as soil-borne fungal pathogens causing root rot or wilting and aboveground pathogens such as *Alternaria solani* in tomato (Linderman 1994; Azcon-Aguilar and Barea 1996; Thygesen et al. 2004; Fritz et al. 2006; Jung et al. 2012; Xiao et al. 2014; Meddich et al. 2015a; Meena and Yaday 2015).

PGPR bacteria promote the growth of plants and represent a beneficial and heterogeneous group of rhizosphere microorganisms on the root surface. They are capable of improving plant growth and increasing tolerance against biotic and abiotic stresses (Dimkpa et al. 2009; Grover et al. 2011; Glick 2012; Oufdou et al. 2014; Dadhich and Meena 2014). PGPR stimulate plant growth through direct mechanisms such as biological nitrogen fixation, phosphate solubilization, stress reduction, and production of phytohormones and siderophores or by indirect mechanisms such as stimulation of mycorrhizal symbiosis, antagonism toward phytopathogens, or removal of phytotoxic substances (Glick 2005; Haas and Défago 2005; Blaha et al. 2006; Couillerot et al. 2009; Zamioudis and Pieterse 2012; Datta et al. 2017a). The mode of action of PGPR is influenced by a number of biotic factors (plant genotypes, plant developmental stages, plant defense mechanisms, other members of the microbial community) and abiotic factors (soil composition, soil management, and climatic conditions) (Vacheron et al. 2013; Meena et al. 2015e; Meena and Lal 2018). Although studies on boosting plant growth through PGPR are widely available, information between the potential uses of PGPR for sustainable development and their present applications remain to be clarified.

The combination of socioeconomic development and population growth in many countries was accompanied by the increase of large quantities of solid and liquid wastes generated mainly by households, green space maintenance services, and industry and farming livestock units (Laarousi et al. 2006; Yadav et al. 2018b; Verma et al. 2015c; Mitran et al. (2018). Despite the fact that organic wastes are full of enormous potential, their use is currently very limited. Morocco, one of the green waste producers, has launched several strategies falling within the framework of sustainable development, which are aimed to preserve the country's natural resources. Therefore, in Marrakesh city, the choice of these green wastes as organic waste is justified by their high abundance in the gardens (18,000 t/year) and their improving effect of the mixture structure by ensuring source carbon for microbial growth. Morocco holds more than 72% of all phosphate rock reserves in the world, being the natural phosphate residue especially phosphogypsum, a mixture of calcium phosphate in various forms and gypsum, never valorized locally. The estimated production of this residue in Morocco is  $20 \times 10^6$  t/year with  $4 \times 10^6$  t of  $P_2O_5$ (El Cadi et al. 2014). Li et al. (2018) and Kammoun et al. (2017) reported that phosphogypsum – as the main by-product of phosphoric acid production – might be

effective in reducing  $NH_3$  and  $CH_4$  emissions throughout the composting process with an increment of  $SO_4{}^{2-}$  content of the compost. Similarly, phosphate sludge waste is generated at significant quantities estimated at  $2 \times 10^6$  t/year.

Currently, animal organic manures like poultry manure are receiving more attention as fertilizers due to the high cost of inorganic fertilizers and its limited ability to improve soil quality for sustainable production systems (Arancon et al. 2008; Kumar et al. 2018b; Meena et al. 2016a). The increase in poultry production driven by the recent demand for low-cholesterol meat products conjunctly with high protein sources and the economic incentive has led to an expansion in the poultry industry worldwide (Sarangi et al. 2016; Dhakal et al. 2015). FAO projections suggest that global meat production and consumption will continue rising over the coming years. Manure from poultry units, containing organic and mineral substances, is often produced in large quantities and discharged into landfills without any exploitation. Other types of waste are generated in significant quantities by industrialists, in particular, liquid effluents (400,000 m³/year) and olive cake (180,000 t/year) (CFC/COI 2008).

In this sense, composting these wastes can be a valuable economic and ecological solution allowing the return of organic matter to the soil as a stable humus-like product and its reintegration to the biogeochemical systems (Francou 2003). Compost is an effective way to increase healthy plant production, reduce costs and the use of chemical fertilizers, and conserve natural resources. Compost provides a stable organic matter that improves the physical, chemical, and biological properties of soils, thereby enhancing soil quality and crop production. Organic amendments are known to improve soil productivity by influencing soil organic matter (SOM) pool. The SOM is considered to be an important criterion of soil quality and therefore is a major determinant of sustainability of agricultural systems. Soil organic carbon (SOC) influences productivity via soil structure, water-holding capacity, soil-buffering capacity, and as a source of plant nutrients. Stable soil structure is in fact required for better soil physical environment. The compost enriches the organic matter in soil with organic molecules, diversified degradation products, and humus substances that improve the belowground structure by interaction with minerals and aggregation of clay particles allowing the production of microaggregates and hence soil stability (Stevenson 1994; Clapp et al. 2001; Seul et al. 2009). This organic matter also decreases the density and promotes the root growth and penetration by improving nutrition, photosynthesis, and plant biomass (Schnitzer and Poapest 1967; Rauthan and Schnitzer 1981; Nardi et al. 1996; Tejada et al. 2009; Varma et al. 2017a; Meena and Yadav 2014). Similarly, the compost increases the cation exchange capacity and soil water retention by ensuring a good flow of water and limiting leaching (Giusquiani et al. 1995; Takeda et al. 2009). It stimulates the activity of microorganisms and thus accelerates the cycle of elements and mineral alteration. The gradual decomposition requires large amounts of macro- and micronutrients necessary for plant nutrition (Clapp et al. 2001). Compost can inhibit the development of pathogenic microorganisms (Tautorus and Townsley 1983, Vassilev et al. 2009; Yadav et al. 2017b). Little information is available about the integrative

potential of microorganisms with organic fertilizers and its effects on crops in the ability of agricultural systems to adapt to climatic and other global changes.

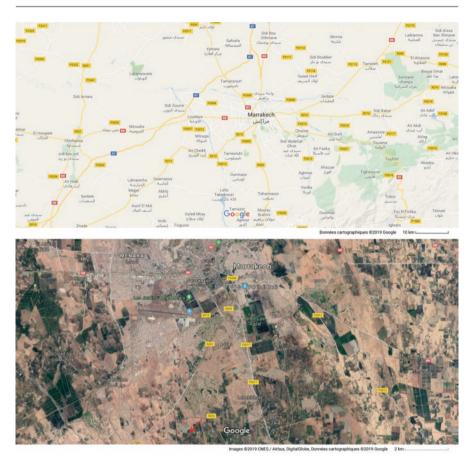
This chapter aims to address the importance of the functional rhizospheric microbiome as a sustainable and effective strategy in plant fitness and disease protection. It also highlights the beneficial interactions among plants and different AMF, PGPR, and compost in boosting agricultural productivity in food security.

Here we will focus on the improvement of the biomass and yield of different agricultural crops – cereals (wheat and corn), vegetable crops (lettuce, tomato, and leek), leguminous (alfalfa), and trees (date palm) – in field via the enrichment of soil by setting up an efficient biological protocol integrating by AMF, exotic and endogenous species; and/or rhizobia, autochthonous bacteria inoculum rhizobia strain; and/or organic soil conditioners resulting from green waste, phosphogypsum, phosphate wash sludge, and agro-industrial poultry waste. This integrated nutrient management approach would improve the fertility of soils and preserve the hydrous resources to reduce the harmful environmental effects while achieving high-quality and high-yielding crops. Also, integrating biofertilizers for the development of plants might be considered as an appropriate strategy to reverse the land degradation trend and encourage sustainable patterns for the development of oasis zones with a vision to promote durable and biological agriculture. Our findings represent the first-of-its-kind study examining the combined application of indigenous/exotic AMF, rhizobia, and organic amendments for the improvement of morphological and physiological parameters, water status, and yields of underlying crops such as leguminous, cereals, and vegetable crops in oasis system. These key species have been selected based on their economic value, their interests and protected status, and their potential for more widespread use by farmers. In addition, our work will illustrate the impact of the interaction effects of the rhizosphere cultivable microorganisms and compost on soil physicochemical properties.

### 9.2 Methodology

### 9.2.1 Study Site

We grew the selected plants in the farm field spread over a total area of 3 hectares and equipped with a drip tape irrigation system. The farm is located in the municipality of Tamesloht, Marrakesh, Morocco (N 31 54 176°; W 008 02 087°; elevation 531 m) (Fig. 9.1). The regional climate of the experimental site is typically Mediterranean, with an average temperature of 20.5 °C and 281 mm of annual rainfall. We didn't apply any herbicides nor chemical fertilizers in the previous growing seasons.



**Fig. 9.1** Geographical location of the study area. (Google Maps 2017)

### 9.2.2 Characterization of Arbuscular Mycorrhizal Fungi

The AMF used were (1) *Rhizophagus irregularis* (pure spores produced in vitro from *Glomus irregularis* (GI) isolate of DAOM 197198), kindly provided by M. Hijri Ph.D. (Research Institute of Plant Biology, University of Quebec, Montreal, Canada), and (2) consortium arbuscular mycorrhizal (CAM) isolated from Tafilalet palms located in Tafilalet, 500 Km southeast of Marrakesh, Morocco (Meddich et al. 2015a). The CAM contains a mixture of endogenous species, *Glomus* sp. (15 spores/gr soil), *Sclerocystis* sp. (9 spores/gr soil), and *Acaulospora* sp. (1 spore/gr soil). The endogenous (native) species were identified according to their color, size, attachment hyphae, and consistency (Koske and Tessier 1983; Morton and Benny 1990).

Fifty (50) of rhizosphere soil samples from 10 to 40 cm in depth – area of date palm tree roots rich with AMF – were collected from Tafilalet palm grove. Samples

were taken at 1 m of palm stems and were spaced 80–100 m. Soil samples were mixed thoroughly to obtain a homogenous sample representative of the entire sampling interval.

The mycorrhizal potential of Tafilalet palm grove was determined by the most probable number (MPN) of propagules per unit of soil method (Plenchette et al. 1989) to reflect the ability of soil to initiate the formation of mycorrhizal associations from propagules, i.e., spores, mycelium, and roots of debris-carrying vesicles.

Corn (*Zea mays* L.) plants were used as a host plant to trap the native mycorrhizal complex naturally associated with date palm and for the multiplication of *G. irregularis*.

MPN propagules were calculated by the following formula: Log MPN =  $(x \log a) - K$ , where x = average mycorrhizal pots, a = dilution factor, and y = s - x where s = dilution number and y is required for the determination of K in the table of Fisher and Yates (1970).

The rate of mycorrhizal root infection was microscopically estimated according to the method described by Trouvelot et al. (1986). The method calculates the parameters of infection as follows:

F: Frequency of root mycorrhization (percentage of root segments infection)

$$F\% = (N - n_0) / N \times 100$$

where N = number of fragments observed and  $n_0 =$  number of fragments without a trace of mycorrhization.

*M: Intensity of cortical infection* (proportion of the cortical colonization in all the mycorrhizal root system)

$$M\% = [(95 \times n_5) + (70 \times n_4) + (30 \times n_3) + (5 \times n_2 + n_1)] / N$$

where  $n_5$ ,  $n_4$ , ...  $n_1$  = number of fragments noted 5,4,... and 1, respectively. Class 5, more than 91%; Class 4, from 51% to 90%; Class 3, from 11% to 50%; Class 2, less than 10 %; Class 1, trace; and Class 0, no mycorrhization.

#### 9.2.3 Characterization of Rhizobacterial Strains

The autochthonous bacterial inoculum, kindly provided by Prof. Oufdou (Cadi Ayyad University, Marrakesh, Morocco), was isolated from the local bean (*Phaseolus vulgaris*) rhizospheric soil – attached to bean roots – and consists of two PGPR and two rhizobia strains. The bacteria selected have been described as plant growth promoters and nitrogen-fixing bacteria. Active culture of strains was prepared in Tryptic Soy Broth (TSB) medium (tryptone, 15 g/L; peptone of soya, 5 g/L;

and sodium chloride, 5 g/L) and agitated for 2–3 days at 28 °C to obtain an optical density (OD = 1) at 600 nm (equivalent to  $10^9$  colony forming unit/ml).

PGPR strains were selected based on their ability for solubilization of complex insoluble phosphate (Raklami 2017). These microbial strains have the ability to solubilize K from K-bearing minerals. These PGPR can produce indole acetic acid (AIA) and exopolysaccharides at very low levels and are incapable of producing hydrogen cyanide (HCN).

# 9.2.4 Preparation of High-Grade Compost by an Enrichment Technique

In our study, the main raw materials used for composting are

- Grass waste (GW) or dandelion
- Dead leaves (DL)
- Waste from livestock units of poultry
- Phosphates washing sludge (GWSP)
- Phosphogypsum
- Pomace olive consisting of olive cake and olive oil mill wastewater (OCOMWWG)
- Household waste

All of the main raw materials were analyzed for physiochemical, nutrients, and heavy metals. Composting was carried out in a composting area consisting of a metal frame of 2400 m at the municipal nursery of Marrakesh. In this experiment, there are five treatments compost piles. The mixtures used for all piles were arranged:

- Grass waste and dead leaves (GWDL)
- Grass waste and phosphates washing sludge (GWSP)
- Pomace olive and household waste (OCOMWWG)
- Phosphogypsum and green waste
- Poultry manure and green waste

The moisture content was maintained at 50–60% by the addition of water throughout the active composting period by frequent checking. To maintain the moisture and prevent excessive loss of heat, drying windrows runoff, and leaching phenomena, the heaps of composting material were then deposited and covered using plastic sheets. The mixtures were turned at 3-day intervals to permit the ventilation, porosity, and high decomposition until the end of the composting. The temperature was measured daily with a thermometer at random depths. The maturity of composts is considered complete when the temperature inside the heap decreased to the surrounding temperature (around 90 days).

# 9.2.5 Inoculation Methods and Growth Under Greenhouse and Field Conditions

Three crops (lettuce, *Lactuca sativa*; tomato, *Solanum lycopersicum*; and leek, *Allium ampeloprasum*), two cereals (wheat, *Triticum aestivum*, and corn, *Zea mays*), one leguminous (alfalfa, *Medicago sativa*), and one tree (date palm, *Phoenix dacty-lifera*) species were tested for the microorganisms and compost effectiveness. The goal was to select plants of economic importance, compatible as date palm underlying culture, and widespread use and capable of producing high biomass under local climatic conditions. Seeds were sterilized in 2.5% sodium hypochlorite, incubated under the corresponding temperature of each plant in the dark condition. They were then placed and cultured on seedling nursery trays and cultured.

Seedlings were transplanted in 5 L pots filled with sterilized soils sampled directly from the research sites to replicate the in situ rhizosphere condition and hence give a better prediction of plant growth promotion effects of AMF and/or PGPR and/or compost for the field trials. Plants were grown under semi-controlled greenhouse conditions; the average temperature was 24.5 °C, average relative humidity was 70%, and light intensity was 330 µmol m<sup>-2</sup> s<sup>-1</sup>. The young palm trees and its underlying cultures were amended by the compost produced locally (Meddich et al. 2017) based on green waste (couch grass, dead leaves), agro-industrial waste (solid and liquid wastes olives), household waste, animal waste (poultry manure), phosphogypsum, and/or phosphate wash sludge. The combination of compost addition and/or AMF and/or PGPR has been evaluated for their growth promotion effect on plants. Furthermore, the physicochemical properties of different waste mixtures and composts were determined.

Under our field experiments, the agricultural soil properties used for plants growth were sandy loam texture (sand, 74.75%; silt, 13.55%; and clay, 11.69%); pH, 8.12; electrical conductivity, 138.3 µs/cm; organic matter, 0.87%; limestone content, 5.04%; phosphorus available, 57.42 ppm; and total nitrogen, 9.98 mg/g. Seedlings were treated by the various biological fertilizers (compost, PGPR, indigenous rhizobia, and/or native or exotic AMF strains).

At transplanting, half of the plants were inoculated (2.8 g) near the root system with the mycorrhizal and disinfected corn roots used as trap AMF (Strullu 1986). The inoculum was infective propagules (mycelium, spores, and roots). A filtrate was added to plants that did not receive the mycorrhizal inoculum (NM plants) in an attempt to restore other soil free-living microorganisms accompanying AMF.

Plants were inoculated two times in different days, 4 ml and 8 ml of each suspension with the symbiotic bacteria PGPR and the rhizobia strains to increase the level of these bacteria in soil and ensure the infection of newly formed roots. The liquid suspension of these strains at a concentration of 10<sup>8</sup> cells/ml for each selected strain was inoculated.

According to our previous studies, we used the low doses of compost (5-10%) in this experiment.

Our field trial was conducted to test the effectiveness of the native biofertilizers as single or co-inoculations on crops biomass. The uninoculated (control) plants for



Fig. 9.2 Field plot layout showing the field design equipped with drip irrigation system

each crop were grown under the same environmental conditions without any biological nor organic amendments. The experimental design was a randomized complete block subdivided into several basic blocks of  $1.5~\mathrm{m} \times 0.8~\mathrm{m}$  each. The plots and their blocks were equipped with a drop by drop system (Fig. 9.2). A device of 12 blocks repeated for the same treatment and the same culture was used to evaluate the impact of the various biological and organic treatments. The evaluation of the yield of the studied crops was determined by measuring the fresh weight, the biomass produced, and/or the number of fruits produced.

#### 9.2.6 Studied Parameters

We evaluated the efficacy of AMF-PGPR and/or compost combination for crop yield production and their impact on soil quality and properties. The AMF infectivity, plant growth, water content, and the physiological parameters for amended and non-amended plants were measured. Nutritional analyses were conducted in treated and non-treated (control) plants.

### 9.2.6.1 Physicochemical Properties of Composts and Soils

Samples were taken at 0–15 cm depth before and after the trial experiment in order to measure the soil physicochemical properties. Field texturing was determined by Robinson's method (Baize 1988). The total organic carbon content was determined

according to Aubert (1978) by the oxidation method of organic material in cold condition with an excess of potassium dichromate  $K_2Cr_2O_7$  in the presence of concentrated sulfuric acid. The total limestone was determined using a Bernard calcimeter. After each brushing waste for composting, the sampling was performed at ten different levels of windrows (deep, surface, side, and center), as described in the method of quartering (AFNOR 1999).

The different soil-size fractions of minerals were determined. The hydrogen peroxide ( $H_2O_2$  to 20 V) was used to remove the organic carbon matter and the sodium hexametaphosphate (50 g/L) for clay dispersion. The portions of coarse sand and fine aggregate were recovered by passing 200  $\mu$ m and 50  $\mu$ m sieve, respectively. The sift-clay fraction was sorted according to Robinson's pipette method. Soil pH was measured by an electrometric procedure using a suspension of 10 g of fresh sample in 20 ml of distilled water. The measurement of bulk soil electrical conductivity (EC) was quantified by a probe. The compost temperature was measured continuously at depth of 30, 70, and 100 cm. Each temperature measurement is an average of six temperature readings taken at three equally spaced locations along the sides of the pile.

Ash content was determined by calcining the previously dried samples in a muffle furnace at 600  $^{\circ}$ C for 6 h. The increase in temperature has been achieved by heat bearing (105  $^{\circ}$ C [1 h], 200  $^{\circ}$ C [1 h], 600  $^{\circ}$ C [6 h]) to prevent from the sudden destruction of the organic matter.

The measurement of the total nitrogen was based on the transformation of organic nitrogen into ammonium nitrogen. After sample mineralization by concentrated sulfuric acid and in the presence of Kjeldahl catalyst, the formed ammonia was displaced by NaOH (40%). Then, the entrained ammonia by the water vapor was fixed by the boric acid and titrated with sulfuric acid. NKT content was determined by the distillation unit Velp-UDK132 according to the protocol described by Rodier (1984).

Ammonium levels were determined according to the Kjeldahl method (AFNOR 1975) from a fresh sample (2 g) using a distillation in an alkaline medium with 10 ml of sodium hydroxide 40%.

Nitrates were measured by passing the filtered sample through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (and reduced nitrate) was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which was measured colorimetrically.

Total phosphorus was determined by a colorimetric assay as described by Olsen and Sommers (1982). The potassium content of the filtered extract was measured using a Jenway PFP7 flame spectrophotometer.

#### 9.2.6.2 Mycorrhization Parameters

Root samples were cleared and stained by trypan blue 0.01% in lactoglycerol (Phillips and Hayman 1970), and mycorrhizal colonization was determined by examining 1 cm root segments (n = 20 per sample) under the microscope. Results were expressed as a percentage of infection (Hayman et al. 1976). The analysis of

the state of the mycorrhizal root system was performed according to the method described by Trouvelot et al. (1986) to characterize the frequency and intensity of mycorrhization in the presence and absence of biofertilizers.

### 9.2.6.3 Measurement of Plant Growth and Minerals Concentration

The response of control and treated plants by biofertilizers was evaluated by determining the shoots (SDM) and root (RDM) dry masses, a reliable indicator of biomass. SDM and RDM were measured after drying the fresh material into the oven at 80 °C until the weight was constant. Mineral determinations (P, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup>) were quantified by a wet digestion method (Pequerul et al. 1993). Dried, finely powered plant samples (0.5 g) were placed in the oven for 6 h at 550 °C. The obtained material was digested in 3 ml of 6N HCl, evaporated on a hot plate, and then recovered with hot distilled water. The solutions obtained were filtered, and the extracts were collected and subsequently stored. The digested solution was shaken gently and filtered through 0.2  $\mu$ m filters (Whatman, England), and the solid fraction was discarded.

The content of phosphorus in the extract was determined according to Olsen and Sommers (1982). The K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup> elements were quantified by a flame photometer (AFP 100 flame photometer). The total content of nitrogen (N) in plants was carried out according to the method described by Kjeldahl.

#### 9.2.6.4 Physiological Parameters

The relative water content (RWC%) of plant leaves was determined according to Sade et al. (2009) as:

$$%WC = (fresh weight - dry weight) / (turgid weight - dry weight) \times 100$$

Turgid weight (TW) was calculated after fully hydrating fresh leaves in darkness at 4°C for 24 h. Results were expressed as percentages.

Leaf water potential (Wh) was measured using a pressure chamber (Scholander et al. 1965). The stomatal resistance was determined in fully expanded leaves of the same rank with an LI-1600 gas exchange system (LI-COR Inc., USA). Chlorophyll fluorescence was measured within plants leaves of the same row using a fluorimeter (OS-30p + OPTI-SCIENCES). The measured parameters correspond to the initial fluorescence ( $F_0$ ), the maximum fluorescence ( $F_m$ ), and the quantum efficiency noted Fv/Fm, where Fv is the variable fluorescence (Tardieu 2005).

### 9.2.7 Statistical Analysis

Values are presented as the mean  $\pm$  standard deviation (SD). Means were tested by one-way analysis of variance (ANOVA) followed by Newman and Keuls test at P < 0.05 in the SPSS software (SPSS Inc., Chicago, IL, USA).

#### 9.3 Results

### 9.3.1 AM Colonization Potential and Infectivity Parameters

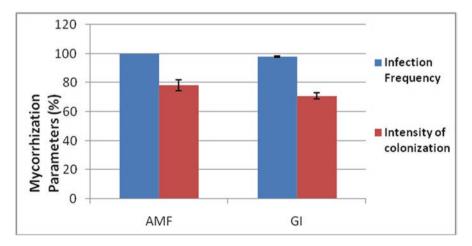
Results of the mycorrhizal roots of corn plants grown on different dilutions of the studied soil are presented in Table 9.1. The symbol (+) represents the plants with at least one point of infection, while the symbol (–) shows the no-infection of roots. For the tested soil, the percentage inoculation with AMF remained 100%, independently of the soil dilution level achieved. The soil of the Tafilalet palm grove shows the most significant number of mycorrhizal propagules, estimated at 1,626.89.

Maize roots inoculated with AMF obtained from Tafilalet palm groves and *Glomus irregularis* (GI) showed higher mycorrhizal frequencies ranging from 98% to 100% (Fig. 9.3). Similarly, the colonization intensity of corn roots remained higher and exceeds 65% after 3 months of cultivation with AMF from palm grove and GI.

	may comment potential of		not p		,		
		Repe	etitio	ıs			
Dilution	Site	$R_1$	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	Number of mycorrhizal plants
1	Palm grove of Tafilalet	+	+	+	+	+	5
1/4		+	+	+	+	+	5
1/16		+	+	+	+	+	5
1/64	-	+	+	+	+	+	5
1/256		+	+	+	+	+	5
1/1024	1	+	+	+	+	+	5

Table 9.1 Mycorrhizal potential of Tafilalet palm grove

R Repetition



**Fig. 9.3** Frequency and intensity of mycorrhizal corn root after 3 months of culture. *AMF* mycorrhizal consortium obtained from Tafilalet palm grove, *GI* Glomus irregularis

# 9.3.2 Composting Agricultural, Animal, and Agro-industrial Wastes

#### 9.3.2.1 Physicochemical Characteristics of Waste Raw Materials

The physicochemical characteristics of the raw materials used in the composting process are presented in Table 9.2 and Fig. 9.4. We found that the pH of the waste varies between 5 and 8, favorable to microorganisms' growth. The dandelions, phosphate sludge, and poultry have an alkaline pH (~8). Poultry manure is rich in total nitrogen (>3%). The tested dandelions and household waste were rich in organic matter and contain relatively moderate amounts of total nitrogen. Most of the waste used in our study presents a C/N ratio between 14 and 40 enough for enhanced microbial activities and suitable for the composting process. Many studies report that pH and C/N ratio are considered important compost parameters owing to their effects on the quality and suitability of the final product for plant growth.

#### 9.3.2.2 Composting Mixture Process

In Fig. 9.5, we illustrate the different phases of composting. The biodegradation can be assessed by the temperature of the compost, which shows a gradual increase during the mesophilic phase and reaching maximum values during the thermophilic phase. For waste mixtures used in our study, we observed that the temperature increased rapidly in the first days of composting reaching maximum values between 56 and 69 °C. Then it gradually decreased to values ranging from 30 to 35 °C approaching the air temperature during the maturation phase.

We evaluated the pH values of the different combination of the main raw material. PMGW had the highest pH values ranged from 8.50 to 9.20, followed by GWDL with values between 7.23 and 8.40. While the lowest pH value was observed in OCOMWWG (5.49–6.06), the compost pH value ranging from 5.5 to 8.5 is considered acceptable (Table 9.3). The combination of grass waste with waste sludge phosphate showed slightly alkaline pH and remained stable throughout the composting process. However, this parameter was increased from 6.50 to 8.00 in PGGW. Monitoring the evolution of C/N ratio in the mixtures GWDL, GWSP,

	Humidity		Total organic	Total Kjeldahl	Ratio
Raw materials	(%)	pН	carbon (%)	nitrogen (%)	C/N
Dandelions (Grass waste)	69.00	8.01	58.10	1.68	34,58
Dead leaves	58.00	6.64	55.40	1.37	40.44
Poultry manure	39.00	8.00	47.30	3.41	13.87
Phosphates washing sludge	59.00	8.29	2.00	0.073	27.39
Phosphogypsum	18.73	5.49	1.64	0.90	2.00
Olive pomace and liquid effluents	54.00	5.77	46.52	1.34	34.72
Household waste (Garbage)	84.40	5.20	60.80	2.41	25.23

Table 9.2 Physicochemical properties of the raw waste before composting



Fig. 9.4 Pictures of the raw material waste used for the composting process and the platform composting

OCOMWWG, PGGW, and PMGW showed a rapid decrease from 33 to 11, 64 to 12, 32 to 19, 47 to 18, and 17 to 12, respectively, after the third month (Table 9.3).

The ash content varies widely among the compost owing to the mineralization of organic matter and concentration of carbon, nitrogen, phosphorus, and other nutrients during composting. Notably, GWSP had the higher ash content relative to other mixtures.

As a result, the composts obtained from grass waste and dead leaves presented the lower levels of available phosphorus, which do not exceed 11 ppm, than those of other compost combinations. The values of this element remained high in the other composts, especially in poultry manure and green waste (1,800 ppm).



Fig. 9.5 Pictures illustrating the different stages during composting

### 9.3.2.3 Compost Characterization at the Maturity Stage

GWDL, GWSP, PGGW, and PMGW had alkaline pH 8.40, 7.77, 8.00, and 9.20, respectively (Table 9.3), but the OCOMWWG stayed neutral or slightly acid (pH = 6.06). The composts with a pH between 6 and 9 are compatible for most plants.

The results of total organic carbon (TOC) have demonstrated that the composts OCOMWWG (37.30%) and PMGW (36.05%) had significantly higher levels than the GWDL (18.40%), GWSP (7.64%), and PGGW (25.00%) (Table 9.3).

The TOC concentration declined for all the treatments between the initial day and the 3 months of the composting period.

One of the often-used criteria to assess the rate of decomposition in the composting process is the C/N ratio since it can reflect the maturity of the compost. The higher C/N ratio at the initial period of compost, C is not in an available form and drops significantly in all the combinations than the end of composting. After 3 months of composting, we found that all composts had lower C/N ratio values ranging from 11.2 (GWDL) to 18.8 (OCOMWWG). The C/N ratio which is less than 20 is indicative of an acceptable maturity and suitable for nursery plant production.

# 9.3.3 Impacts of Biofertilizers on Growth and Physiological Parameters of Date Palm (*P. dactylifera*) and the Underlying Crops

#### 9.3.3.1 The Case Study of Date Palm

# 9.3.3.1.1 Effects of Biofertilizers on the AMF Infectivity, Growth, and Water Status of Date Palm

We assessed the effects of the single or combination application of compost (5%) and AMF (mycorrhizal consortium of Tafilalet) on *P. dactylifera* growth. As a result,

Table 9.3 Physicochemical parameters during composting

Composting stage	Hd	TOC (%)	TKN (%)	C/N Ratio	Ashes (%)	P (mg/g)
GWDLi	$7.23 \pm 0.13$	$41.60 \pm 1.66$	$1.25 \pm 0.24$	$33.28 \pm 1.02$	$19.00 \pm 1.46$	$0.009 \pm 0.0010$
GWDL3	$8.40 \pm 0.08$	$18.40 \pm 1.01$	$1.60 \pm 0.32$	$11.20 \pm 0.31$	$31.54 \pm 2.43$	$0.011 \pm 0.0012$
GWSPi	$7.86 \pm 0.18$	$25.00 \pm 1.37$	$0.39 \pm 0.08$	63.77 ± 2.35	$50.00 \pm 2.11$	$0.167 \pm 0.019$
GWSP3	$7.77 \pm 0.14$	$7.637 \pm 040$	$0.61 \pm 0.16$	$12.39 \pm 0.46$	$82.00 \pm 3.47$	$0.300 \pm 0.035$
OCOMWWGi	$5.49 \pm 0.04$	$50.50 \pm 2.74$	$1.57 \pm 0.35$	$32.16 \pm 1.37$	$3.90 \pm 0.30$	I
OCOMWWG3	$6.06 \pm 0.05$	$37.30 \pm 2.05$	$1.98 \pm 0.40$	$18.84 \pm 0.85$	$5.60 \pm 0.43$	1
PGGWi	$6.50 \pm 0,06$	$35.33 \pm 1.89$	$0.75 \pm 0.15$	$47.10 \pm 1.42$	$35.56 \pm 2.76$	$0.500 \pm 0.057$
PGGW3	$8.00 \pm 0.10$	$25.00 \pm 1.36$	$1.40 \pm 0.29$	$17.85 \pm 0.62$	44.80 ± 3.39	$0.210 \pm 0.024$
PMGWi	$8.50 \pm 0.17$	$44.86 \pm 2,26$	$2.59 \pm 0.53$	$17.32 \pm 0.41$	$19.25 \pm 1.41$	$1.780 \pm 0.14$
PMGW3	$9.20 \pm 0.21$	$36.05 \pm 1.96$	$2.87 \pm 0.61$	$12.56 \pm 0.38$	$35.30 \pm 1.89$	$1.800 \pm 0.20$
GWDL orass waste and deg		grass waste and sludge of	of phosphate OCOM	WWG olive cake olive	oil mill wastewater a	d leaves. GWSP orass waste and studoe of phosphate. OCOMWWG olive cake olive oil mill wastewater and oarbaoe. PGGW phos-

GWDL grass waste and dead leaves, GW3P grass waste and sludge of phosphate, OCOMWWC olive cake olive oil mill wastewater and garbage, PGCW pnosphogypsum and green waste, and PMGW poultry manure and green waste, i initial, 3 months of composting and 6 months, TOC total organic carbon, TKN: total Kjeldahl nitrogen, P available phosphorus

the frequency of infection (F) of palm root system with AMF remained high (F > 60%), and it was substantially affected by the application of compost (Table 9.4). Plants inoculated with AMF produced higher above- and belowground biomasses than the control plants. The single application of the organic amendments has no impact on the root growth of the palm. Interestingly, the combination AMF + green waste compost had significantly higher shoot and root biomasses, relative water content, and water potential than the no-treated control plants. Indeed, our results showed that the association AMF and compost GWDL increased  $1.6\times$  shoot and  $1.9\times$  root biomasses than the control.

Moreover, amended plants with AMF+compost showed a relative water content (81%) slightly higher to the control (77%) (Table 9.4). The mycorrhizal date palms and amended with compost had a higher leaf water potential (-16.83 bar) than control plants (-30.37 bar). Exposure of date palm to the single AMF (2.21 s/cm) or compost (2.22 s/cm) or combined (2.12 s/cm) led to a considerable decrease in stomatal resistance (R) than no-treated plants (2.93 s/cm).

## 9.3.3.1.2 Date Palm (*P. dactylifera*) Treated with AMF and/or Compost Showed Increased Minerals

We assayed the nutrient contents in shoots of date palm leaves amended with AMF and/or compost since the degree of growth depends on their uptake and translocation. Shoot N, P, K, Ca, and Mg was significantly higher in plants treated with single or synergism effect of AMF and compost than control plants (Table 9.5). These results could at least partly be explained by the effective contribution of mycorrhizal association in improving nutrients of plants through the development of fungal hyphae, allowing good use of the soil minerals and their mobilization to the plants. The positive effects of applying compost GWDL on mineral nutrition was clearly observed. The values of the ionic content are higher in palms amended with AMF+compost than control plants.

### 9.3.3.2 The Case Study of the Underlying Crops

#### 9.3.3.2.1 Impacts of Biofertilizers on AMF Infectivity and Crop Growth

The calculated frequency of mycorrhization in alfalfa, tomato, wheat, and corn roots exceeds 90% and that in the absence of compost (Table 9.6). On the contrary, the rate of mycorrhization decreases and remains below 66% for plants amended with compost.

#### 9.3.3.2.2 Effect of GWDL Compost and AMF on Alfalfa Biomass

After 2 months of culture, the application of GWDL at doses of 5% has a beneficial effect on improving the production of the shoot and root dry biomasses of alfalfa (*Medicago sativa*) than the control (Fig. 9.6 and Table 9.6). The combination compost+AMF showed the highest values of the shoot and root biomasses.

Table 9.4 Impact of biofertilizers on growth, water status, and stomatal resistance of date palm after 4 months of culture

•						
Treatment/	Frequency of			Relative water content   Water potential	Water potential	Stomatal
combination	mycorrhization (F) (%)	orrhization (F) (%)   Aerial dry mass (g)   Root dry mass (g)   (RWC) (%)	Root dry mass (g)	(RWC) (%)	Ψh (bar)	resistance R (s/cm)
Control plants	I	$0.407 \pm 0.015$	$0.116 \pm 0.016$	$77.21 \pm 2.78$	$-30.37 \pm 0.70$   $2.93 \pm 0.10$	$2.93 \pm 0.10$
Compost GWDL	I	$0.448 \pm 0.015$	$0.088 \pm 0.007$	$80.75 \pm 2.32$	$-24.07 \pm 0.98$ $2.22 \pm 0.13$	$2.22 \pm 0.13$
AMF	$74.29 \pm 5.05$	$0.460 \pm 0.020$	$0.128 \pm 0.007$	$79.10 \pm 2.08$	$-27.57 \pm 0.40$   $2.21 \pm 0.15$	$2.21 \pm 0.15$
GWDL + AMF	$ 62.89 \pm 3.77 $	$0.670 \pm 0.010$	$0.216 \pm 0.020$	$81.65 \pm 2.02$	$-16.83 \pm 1.44$   2.12 ± 0.11	$2.12 \pm 0.11$

GWDL grass waste and dead leaves, AMF mycorrhizal consortium of Tafilalet

**Table 9.5** Effects of mycorrhization and/or compost on the mineral composition of date palm after 4 months of culture

Element	Treatments	Content (mg/g DM)
N (mg/g DM)	Control	$12.14 \pm 0.452$
	AMF	18.64 ± 0.516
	Compost GWDL	$20.54 \pm 0.935$
	Compost GWDL + AMF	$26.33 \pm 0.539$
P (mg/g DM)	Control	$3.73 \pm 0.428$
	AMF	$3.62 \pm 0.436$
	Compost GWDL	$5.44 \pm 0.180$
	Compost GWDL + AMF	$7.98 \pm 0.921$
K (mg/g DM)	Control	$1.49 \pm 0.231$
	AMF	$1.79 \pm 0.141$
	Compost GWDL	1.85 ± 0.107
	Compost GWDL + AMF	2.01 ± 0.104
Ca (mg/g DM)	Control	$0.55 \pm 0.127$
	AMF	$0.86 \pm 0.032$
	Compost GWDL	$1.03 \pm 0.168$
	Compost GWDL + AMF	$1.15 \pm 0.078$
Mg (mg/g DM)	Control	$1.49 \pm 0.122$
	AMF	1.77 ± 0.1417
	Compost GWDL	$2.07 \pm 0.065$
	Compost GWDL + AMF	$2.74 \pm 0.097$

 $\mathit{GWDL}$  grass waste and dead leaves,  $\mathit{AMF}$  mycorrhizal consortium of Tafilalet palm grove,  $\mathit{DM}$  dry matter

# 9.3.3.2.3 Tomato Has Better Growth After the Compost (GWSP) and/or AMF Applications

We evaluated tomato growth parameters after the amendment with mycorrhizal consortium Tafilalet (AMF) and/or GWSP compost (a mixture of dandelions and phosphates washing sludge, at 10% dose) (Table 9.6). The production of the shoot and root biomasses has increased following the colonization with AMF and/or compost, being more relevant after the combination of both treatments. Indeed, the synergism effects of both biofertilizers have markedly improved plant growth compared with uninoculated control.

# 9.3.3.2.4 Wheat Plants Showed Increased Biomass in Response to Compost (OCOMWWG) and/or AMF

The application of compost-based waste pomace olive and garbage (OCOMWWG) at a dose 10% or the inoculation of the roots by *Glomus irregularis* (GI) increased slightly the above- and belowground biomasses of wheat than control plants (Table 9.6). The dual application of GI and OCOMWWG compost has no positive effect on the SDM of wheat, but this combination has increased the root biomass than control plants.

			Frequency of mycorrhization	Aerial dry	Root dry mass
Plants		Treatments	(F) (%)	mass (g)	(g)
Legume	Alfalfa	Control plants	_	$0.006 \pm 0.00$	$0.006 \pm 0.004$
		Compost GWDL	_	$0.379 \pm 0.01$	$0.234 \pm 0.029$
		AMF	$100.00 \pm 5.77$	$0.079 \pm 0.03$	$0.075 \pm 0.025$
		Compost GWDL + AMF	$52.00 \pm 7.23$	$0.666 \pm 0.05$	$0.361 \pm 0.051$
Vegetable	Tomato	Control plants	_	$0.060 \pm 0.01$	$0.020 \pm 0.003$
crops		Compost GWSP	_	$0.130 \pm 0.01$	$0.030 \pm 0.003$
		AMF	91.55 ± 4,57	$0.100 \pm 0.00$	$0.030 \pm 0.002$
		Compost GWSP + AMF	$65.25 \pm 5,57$	$0.350 \pm 0.02$	$0.100 \pm 0.007$
Cereals	Wheat	Control plants	-	$0.068 \pm 0.01$	$0.004 \pm 0.001$
		Compost OCOMWWG	_	$0.082 \pm 0.01$	$0.018 \pm 0.002$
		GI	95.00 ± 3,84	$0.077 \pm 0.01$	$0.017 \pm 0.001$
		Compost OCOMWWG+ GI	$60.00 \pm 3,85$	$0.065 \pm 0.01$	$0.013 \pm 0.003$
	Corn	Control plants	-	$0.438 \pm 0.03$	$0.139 \pm 0.013$
		Compost OCOMWWG	_	$0.648 \pm 0.06$	$0.137 \pm 0.036$
		GI	98.04 ± 3,85	$0.534 \pm 0.04$	$0.241 \pm 0.02$
		Compost OCOMWWG + GI	$65.77 \pm 5.05$	$0.456 \pm 0.08$	$0.183 \pm 0.013$

**Table 9.6** Impacts of biofertilizers on the growth of underlying crops

GWDL grass waste and dead leaves, GWSP grass waste and sludge of phosphate, OCOMWWG olive cake, olive oil mill wastewater, and garbage, AMF mycorrhizal consortium of Tafilalet, GI Glomus irregularis

# 9.3.3.2.5 Compost (OCOMWWG) and AMF Promote Aerial and Root Traits of Corn Plants

The mixed treatment of corn with compost (OCOMWWG) and AMF (*G. intraradices*) increased shoot and root dry matters. In the same line of results, the single inoculation of the corn plant with GI improved the 1.25× the aerial biomass and 1.71× the root DM. However, we observed no difference of the mentioned parameters between the efficiency of compost alone on the belowground biomass.

# 9.3.4 The Potential Effects of Biofertilizers to Improve Crops Yield in the Field

We evaluated alfalfa, green and red lettuces, leek, and wheat treated with AMF (*G. intraradices*)-rhizobia and/or compost (poultry manure and green waste) in the field to compare the yield trait with those of untreated control plants. Each crop was



**Fig. 9.6** Effect of mycorrhizal consortium of Tafilalet (AMF) and grass waste and dead leaf (GWDL) compost on alfalfa growth after 2 months of culture



**Fig. 9.7** Implementation and randomization of crops plantations treated or not with different biofertilizers (AMF, PGPR, and/or compost)

randomly arranged in the different blocks of the managed parcels (Fig. 9.7). We found a significant difference in yielding after biological and organic fertilizers uses in leguminous (alfalfa), vegetable crops (lettuce, leek), and cereals (wheat) (Table 9.7). The application of indigenous AMF-rhizobia and compost (GWDL) increased 2× the total fresh biomass of alfalfa and green lettuce than control plants.

Composts (PMGW) promoted 4.4× the yield in leek plants and  $1.5\times$  red lettuce. The synergism of composts enriched by PGPR and *G. irregularis* improved yields of red lettuce and leek  $1.6\times$  than control plants. The single application of PGPR or its combination with compost and *GI* increased significantly red lettuce yield.

We also examined the important positive effects of the combination of AMF-PGPR and rhizobia on wheat yield compared to untreated control plants.

	Plant	Treatment/combination	Yield (g/plant)
Legume	Alfalfa	Control plants	11.63 ± 1.15
		Compost GWDL	14.93 ± 3.22
		AMF	17.46 ± 2.26
		Rhizobia	16.53 ± 2.97
		Compost GWDL + AMF + rhizobia	26.06 ± 3.25
Vegetable crops	Green lettuce	Control plants	357.10 ± 43.28
		Compost PGGW	464.75 ± 65.44
		GI	536.75 ± 64.49
		Compost PGGW + GI	$635.38 \pm 90.27$
	Red lettuce	Control plants	465.60 ± 32.95
		Compost PMGW	685.15 ± 59.68
		GI	557.15 ± 40.78
		PGPR	$739.10 \pm 43.33$
		Compost PMGW + GI + PGPR	$761.57 \pm 35.07$
	Leek	Control plants	$5.27 \pm 1.53$
		Compost PMGW	$23.00 \pm 5.49$
		GI	$6.32 \pm 0.57$
		PGPR	$6.28 \pm 0.66$
		Compost PMGW + GI + PGPR	$9.00 \pm 1.28$
Cereal	Wheat	Control plants	$4.52 \pm 0.18$
		AMF	$5.73 \pm 1.03$
		PGPR	$5.45 \pm 0.39$
		Rhizobia	$7.37 \pm 1.12$
		AMF+PGPR+rhizobia	11.00 ± 1.16

**Table 9.7** Impacts of the tested biofertilizers on crops yield in the field

n=12 belonging to each of the 12 repeated blocks for the same treatment and the same culture GWDL grass waste and dead leaves, PGGW phosphogypsum and green waste, PMGW, poultry manure and green waste, AMF mycorrhizal consortium of Tafilalet, GI Glomus irregularis, PGPR plant growth-promoting rhizobacteria

These results suggest the beneficial role of the tripartite association AMF-PGPR-compost to increase the yield of underlying crops. This efficacy depends on the plant cultivar, the nature, and dose of the compost and the mycorrhizal and bacterial strains used.

# 9.3.5 Assessment of Physicochemical Parameters in Soil Samples Collected from the Agricultural Areas

We evaluated physicochemical properties of the field soils used before and after the experimentation (Table 9.8). The percentages of sand (74.75%) and silt (13.55%) were higher compared to the other soil elements. The soil used for our field trial is classified as calcareous with a pH value of 8.12. The soil conductivity was ranging between 0 and  $500 \,\mu\text{s/cm}$ , and the percentage of limestone was 5.04%. In addition,

Table 9.8 Physicochemical properties of agricultural soil before and after the plantation

•			•				
			Conductivity	Total carbon	Organic	Total nitrogen	Available
Date	Analyses	pH	(µs/cm)	(%)	matter (%)	(mg/g)	phosphorus (ppm)
Before the experiment	Soil (start	$8.12 \pm 0.06$	$138.30 \pm 2.91$	$0.50 \pm 0.04$	$0.87 \pm 0.15$	$9.98 \pm 0.95$	$57.42 \pm 1,21$
	experiment)						
At harvest time cereals	Control	$8.05 \pm 0.04$	$506.66 \pm 5.82$	$1.22 \pm 0.07$	$2.10 \pm 0.13$	$18.20 \pm 1.92$	$57.00 \pm 1,61$
(wheat)	AMF+	$7.38 \pm 0.03$	$204.50 \pm 3.65$	$2.30 \pm 0.11$	$3.97 \pm 0.19$	$30.80 \pm 2.87$	$63.20 \pm 4.33$
	PGPR+rhizobia						
At harvest time legume	Control	$8.36 \pm 0.08$	$150.00 \pm 2.29$	$0.79 \pm 0.05$	$1.20 \pm 0.10$	$23.98 \pm 2.07$	$61.40 \pm 1,05$
(alfalfa)	Compost GWDL +	$8.25 \pm 0.05$	$210.00 \pm 4.34$	$1.19 \pm 0.03$	$2.05 \pm 0.17$	$42.65 \pm 3.77$	$74.77 \pm 3.95$
	AMF+rhizobia						
At harvest time	Control	$7.52 \pm 0.18$	$257.00 \pm 6.45$	$0.64 \pm 0.10$	$1.40 \pm 0.18$	$8.07 \pm 1,62$	$46.71 \pm 1,65$
vegetable crop (leek)	Compost PMGW+	$7.55 \pm 0.08$	$246.00 \pm 5.54$	$0.95 \pm 0.06$	$1.92 \pm 0.11$	$9.93 \pm 1,62$	$195.86 \pm 7,22$
	GI +PGPR						
At harvest time	Control	$7.49 \pm 0.03$	$424,00 \pm 3.32$	$1.11 \pm 0.06$	$2.42 \pm 0.10$	$9.93 \pm 1,62$	$106.16 \pm 6.77$
vegetable crop (red and	Compost PMGW+	$7.72 \pm 0.02$	$237.00 \pm 6.55$	$1.64 \pm 0.06$	$2.18 \pm 0.10$	$9.23 \pm 1,60$	$257.43 \pm 12,18$
green lettuces)	GI +PGPR						

GWDL grass waste and dead leaves, PMGW poultry manure and green waste, AMF mycorrhizal consortium of Tafilalet, GI, G. irregularis, PGPR plant growthpromoting rhizobacteria

the contents of total carbon (0.50%) and organic matter (0.87%) reflected the poorly remineralized soil organic matter. Furthermore, the field soil before starting the treatments contains 9.98 mg/g of total nitrogen and 57.42 ppm of available phosphorus.

After the field trials, our results (Table 9.8) showed that the treatments applied (compost, AMF, and PGPR) have improved soil quality than the before starting. Moreover, our treatments have slightly decreased the value of pH, with the exception of leguminous. The electrical conductivity has increased throughout the cultivation, being the untreated cereals (506.66 μs/cm) and lettuces (424 μs/cm) the highest. Relative to untreated control conditions, the biofertilizers increased total organic matter and total carbon content especially the combination AMF-PGPR-compost. The application of tripartite combination was correlated positively with the organic matter (3.97%) and total carbon (2.30%) in wheat crops. The total nitrogen content in soil was also enhanced by the application of biofertilizers. The highest value of total nitrogen (42.65 mg/g) was observed in alfalfa plants grown under the amended condition of AMF-rhizobia-compost. This element remained similar to the value obtained at the initial in leek and lettuce independently of the treatment.

Interestingly, the application of biofertilizers improved the soil available phosphorus at the harvest of different cultures, being the highest values observed in the rhizosphere of lettuce  $4.5 \times (257.4 \text{ ppm})$  and leek  $3.41 \times (195.8 \text{ ppm})$  compared to control (57.4 ppm).

#### 9.4 Discussion

The purpose of our study was to investigate the growth promotion effect of single and combined (1) compost-based crop residues and animal wastes, microorganisms, (2) AMF (native AMF, mycorrhizal consortium of Tafilalet, and exotic AMF, G. intraradices), and (3) rhizobia and indigenous PGPR isolated from soil of the research sites. All these key players were tested in the greenhouse and the field for their effect on biomass, yield, development and physiology, and nutrient levels in several crop tissues. The mycorrhizal potential of a soil depends on the number of spores present in the rhizosphere, their quality, and capacity for adaptation and infectious properties. For instance, the number of mycorrhizal propagules of Tafilalet palm groves rhizosphere (1627/100 g) is 7.5× higher than palm grove northeast of Marrakesh in Morocco (219 propagules/100 g) (Meddich et al. 2017; Meena et al. 2014); Varma et al. 2017b; Kumar et al. 2018a). Whereas the mycorrhizal potential of saline soils of the Marrakesh palm grove does not exceed 149 propagules per 100 g of soil (Meddich et al. 2015c; Meena et al. 2015c; Yadav et al. 2018a). Changes in physicochemical properties of rhizospheric soil such as soil pH, water potential and partial pressure of O<sub>2</sub>, and plant exudation could affect the ability of PGPR strains to colonize the rhizosphere.

The infectivity parameters (F% and M%) were higher in Tafilalet palm groves soils than the reference strain *G. irregularis*. The consortium mycorrhizal isolated from Tafilalet oasis area and selected *G. irregularis* showed a great ability to infect

palms roots and the underlying crops (wheat, corn, alfalfa, leek, lettuce, and tomato). These results suggest the presence of variability in the parameters of infectivity of AMF according to the host plants and the conditions of the medium, A signal exchange between the two partners AMF-plant could be established, and molecules contained in the root exudates influence the development of the arbuscular mycelia (Gianinazzi-Pearson et al. 1996; Meena et al. 2018b; Dadhich et al. 2015; Sofi et al. 2018). Subsequently, AMF mycelia colonize cortical cells and give rise to fungal arbuscules representing the preferred site of exchange between the fungus and the host plant (Gianinazzi-Pearson and Gianinazzi-Silvio 1988; Gianinazzi-Pearson et al. 1996). The frequency of mycorrhization of palm roots and underlying crops with Tafilalet consortium and G. intraradices decreased following the application of compost. This could be due, at least partly, to the richness of compost in mineral elements or the high water retention inhibiting, by asphyxiation, thus the development of the symbiotic association and undermining the aggressivity of the mycorrhizal isolates. In addition, plants subjected to these conditions can directly benefit from the organic and mineral amendments and the absorption of water without establishing the relationship with AMF. Similar results were reported by Meddich (2001) for clover and barley imposed to increasing concentrations of mineral elements, especially phosphorus.

It is important to note the importance of mixed inoculation of AMF and compost in improving the growth and mineral nutrition of date palm. Mycorrhizal and amended palms with compost showed higher levels of N, P, K, Ca, and Mg than control plants, suggesting the compost and bacterium's ability to increase crops absorption of minerals.

The increase of waste temperature during the aerobic process of composting owing to the metabolism of microorganisms to solubilize the organic compounds. Hachicha et al. (2009) reported that during composting, a temperature exceeding 60 °C and maintained for several days ensures the destruction of pathogenic microorganisms. Generally, four phases of temperature fluctuation exist in the composting process: mesophilic, thermophilic, cooling, and maturation. The decrease in temperature during the maturity phase owing to the depletion of easily biodegradable organic matter (Gea et al. 2003; Petiot and Guardia 2004; Meena et al. 2016b; Sihag et al. 2015; Verma et al. 2015a).

The increase in pH can be explained by the accumulation of ammonia and/or a loss of short-chain fatty acids and volatiles resulting from the microbial activity (Lim et al. 2012; Shak et al. 2014). The rapid decrease in C/N observed in our compost at the third month of composting phase could be explained by the significant reduction in the metabolizable organic carbon related to the biodegradation of organic matter. Compost with a C/N ratio below 20 is considered mature and can be used without any restrictions (Jimenez and Garcia 1989). A C/N ratio close to 10–15 is often considered as an index of humic material formation and stability of composts (Lim et al. 2014; Meena et al. 2015a; Yadav et al. 2017a; Kakraliya et al. 2018). The application of low dose (5%) of compost GWDL, with low levels of available phosphorus, with AMF isolated from Tafilalet palm grove, showed a beneficial effect to improve *P. dactylifera* growth parameters. Roca-Pérez et al. (2009)

reported that the addition of organic matter by adding compost improves soil structure, fertility, porosity, and water retention. Several soil properties, including structure and porosity, affect root growth (Roca-Pérez et al. 2009; Datta et al. 2017b; Ram and Meena 2014). The high content of organic matter in composts stimulates the biological and enzymatic activities of substrates and the bioavailability of nutrients by mineralization of the organic matter (Hofman and Dušek 2003; Crecchio et al. 2004; Meena et al. 2015b). Also, the humic substances might promote nutrient uptake and can determine the rhizogenic activity (Eyheraguibel et al. 2008).

Overall, the low dose (5% and 10%) of the tested compost with or without mycorrhizal fungi has beneficial impacts on improving the growth of crops species: alfalfa, tomato, wheat, and corn. Similar results were observed in lettuce and maize amended with low compost concentrations (Mrabet et al. 2011). In contrast, the negative effect of 100% compost dose application has decreased both aerial and root biomasses and nutrients uptake than control (Meddich et al. 2017; Dhakal et al. 2016; Kumar et al. 2017a). This finding owing to the high concentration of mineral and elements in the substrate leading to inadequate assimilation of nutrients. Similar results were found in corn plant biomass amended with high compost concentration (Abouelwafa 2009).

Indeed, our study showed that the interaction of low-dose compost GWDL (5%) and AMF has significantly stimulated shoot and root biomasses of alfalfa. Similar results were observed in tomato plants treated with the combination of the low dose 10% of compost GWSP and AMF. The single application of GI and OCOMWWG promoted the growth of wheat and corn, while the combination GI+OCOMWWG has positively affected the root growth. These results confirm the good functioning of mycorrhization under limiting conditions and soils with low organic matter and nutrients supply (Meddich et al. 2017).

The application of different doses of phosphorus or NPK chemical fertilizers to substrates for growing non-mycorrhizal plants of clover, barley, and date palm did not lead to better results to those obtained with AMF or compost application (Meddich 2001; Meddich et al. 2015d). The availability and mobilization of phosphorus element with AMF or composts could not be solely responsible for improving plant tolerance to water and salt stress. Other nutrients such as Ca, K, N, and Mg could contribute to these strategies. Also, a better distribution of the water circulation in the plant can explain, partially, this tolerance in presence of AMF.

Our genetic analyses revealed the expression of three genes of MIP family coding for the synthesis of aquaporins in mycorrhizal clover roots with the Aoufous complex of Tafilalet and *G. monosporus* under severe drought stress (30% FC) (Zeze et al. 2007, 2008; Meena et al. 2017a). In this study, *P. dactylifera* inoculated with AMF or amended with compost showed similar RWC than control plants, whereas mycorrhizal palms amended with compost showed higher leaf water potential than control plants. At the same time, mycorrhizal and treated plants with compost showed the lowest stomatal resistance compared to control plants. The low stomatal resistance in amended plants could improve the mesophilic CO<sub>2</sub> uptake (Brown and Bethelenfalvay 1987) conferring an increase in photosynthesis (Lawlor 1987).

To assess the efficacy of microorganisms and compost on the underlying culture used in arid and semiarid regions, we exposed several crop species to the application of AMF-rhizobia and compost (GWDL). Our field assessment results indicate yield enhancement of wheat and alfalfa. Our results corroborate findings in *Vicia faba* (Jia et al. 2004), beans (Amrani 2009) and *Vigna unguiculata* (Clautilde et al. 2011) inoculated with rhizobia and AMF. The interactions between plants and AMF and/or rhizobia bacteria by which all partners could benefit from the mutual association may improve the growth of the plants owing to the mechanisms of growth promotion developed by the microorganisms such as the fixation of nitrogen, mineral solubilization, water uptake, and phytohormone production (Finlay 2007; Jalili et al. 2009; Oufdou et al. 2014; Verma et al. 2015b).

We also assessed the yield traits of underlying crops under the exposition to agricultural, animal, or agro-industrial wastes. Compost of green waste associated with poultry manure (PMGW) has considerably increased the yield of leek and red lettuce. The combination of this compost enriched with PGPR and G. irregularis improved also the yield of red lettuce. Our results are comparable to those obtained by Koulibaly et al. (2015) showing an increment of 65% of the cotton plant yield after the addition of compost. Copetta et al. (2011) showed that the use of AMF and compost from green waste considerably improved the yield and quality of tomato fruit. Composts improve the different physicochemical and biological properties of soils (Toumpeli et al. 2013; Mehta et al. 2014; Meena et al. 2017c) and consequently increase the yield of plants (Motta and Maggiore 2013). They are able to improve the mineral and water status of plants (Gharib et al. 2008; Meddich et al. 2015c; Layek et al. 2018). In addition, compost enriched soil with organic matter and microorganisms. These components contribute to make available and store nutrients for plants, promote the biological activity as a source of energy for microorganisms, and help on the structure, physicochemical properties, and aeration of the soil. Furthermore, the composts are involved in the maintenance of sandy soils and colloidal particles to avoid the erosion phenomena by retaining the particles set in motion by the rain and absorbing the drops (Bodet and Carioli 2001).

PGPR have the ability to solubilize complex phosphate, assimilate nitrogen, and reduce stresses by modulating the expression of ACC deaminase (Jalili et al. 2009). They are able to modulate the growth and architecture of crop roots by releasing phytohormones (i.e., auxin, cytokinins, etc.) or other antimicrobial and/or antifungal substances for the control of the harmful effects of pathogens (Souza et al. 2015). The pathway of AIA synthesis by PGPR could also stimulate plant growth (Barnawal et al. 2012; Chen et al. 2013; Meena et al. 2017b).

It is notable that AMF can solubilize phosphate and mobilize other nutrients for the benefit of the plant (Jia et al. 2004; Clautilde et al. 2011; Tarraf et al. 2017). Furthermore, AMF have the ability to improve the water status of plants (Zeze et al. 2007, 2008; Baslam et al. 2013). They are capable of mobilizing macro- and micro-elements in soil and water level in plants and controlling pathogens.

The agricultural soil analysis carried out before the plantation was able to characterize the sandy loam texture, low in organic matter (0.87%), low electrical conductivity (138.3  $\mu$ s/cm), and slightly alkaline pH (8.12) owing to the high

limestone content (5.04%). Moreover, the phosphorus content available from the soil (57.42 ppm) is relatively low, which is in favor of the formation, development, and proper functioning of symbiosis between plants and microorganisms such as PGPR and AMF (Meddich et al. 2015d, 2017). The application of compost can be of great interest in improving the fertility of agricultural soils and consequently improving crops growth and yield (Meddich et al. 2016; Meena et al. 2018a).

The AMF applied in our study were infectious and adapted to all the studied crop species showing their higher frequency of mycorrhization in seedlings treated with the association with compost. AMF infectivity and root colonization rates are positively correlated to improve crop biomass and plant physiological and water parameters (Meddich et al. 2015a, b). Sghir et al. (2014) observed that mycorrhizal frequencies of date palm roots and arbuscular contents decreased significantly in palm trees inoculated with the combination AMF-Trichoderma harzianum than in plants inoculated with only AMF. However, the double inoculation makes a major contribution to the growth and root architecture of date palm (Sghir et al. 2014; Meena et al. 2018c) and high yielding of soybean (Egberongbe et al. 2010). This suggests the existence of compromises and positive and complementary impacts between the symbiotic microorganisms and their host plant. The physicochemical properties of the agricultural soil after the crop harvests showed that all treatments had a positive effect on the nutritional and physicochemical properties of the rhizosphere. In fact, the contents of organic matter, carbon, and available phosphorus improved by the composts and/or microorganisms compared to uninoculated control soil. According to Caravaca et al. (2002), the mycorrhizal inoculation of Olea europaea was very effective in improving soil quality. Other studies (Bhattacharyya and Jha 2012; Sharma et al. 2013) have found that the ability of microorganisms especially PGPR and rhizobia improved the quality of soil and the availability of nutrients through different mechanisms including solubilization of phosphate and potassium, symbiotic and free nitrogen fixation, and the production of siderophores.

Together the results of field trials suggest that indigenous biofertilizers can constitute a better alternative well adapted to the use of chemical fertilizers in arid and semiarid conditions and can fulfill diverse beneficial interactions in plants leading to promising solutions for sustainable and environment-friendly agriculture.

### 9.5 Future Perspectives

Healthy soil is vital to life on Earth to maintain or increase the global yield production by at least 70% to feed the anticipated  $9.6 \times 10^9$  people by 2050. Yield losses are caused by the effects of climate change and by indirect effects such as increased inputs in crop production. To counteract these negative effects, various adaptation strategies have been suggested. Benefiting the soil in terms of quality or health is closely linked by the adoption of best management practices. These principles call for the integrated use of beneficial microorganisms and organic manures to meet global food security and sustainable agriculture demands. Thus,

this study clearly pointed out how the natural microbial-mediated process can impact positively the soil and consequently growth and yield of crops adapted to harsh environmental conditions. Our approach of rhizoengineering based on the single or multi-inoculation of native or exotic microorganisms such as AMF and PGPR, together with the use of different compost-based growing media, influences the nutrient use of plants and the rhizosphere quality. An understanding of the mechanisms of action of these complex interactions of the compost and/or microbial-promoted increase of crop yield and health and soil fertility has yet to be explored. Further, future researches hinging soil aspects in addition to the primary focus of crop yields are needed. At same time, long-term agronomic experiments in different agro-ecological zones across the world to provide practical datasets pertinent to soil quality are time-demanding tasks.

#### 9.6 Conclusions

In summary, our results demonstrate:

- The soils of Tafilalet palm grove showed higher mycorrhizal potential and infectivity capacity. The mycorrhizal fungi isolated from this grove and *G. irregularis* were infectious and increased the biomass and other physiological parameters of the date palm and its underlying crops (wheat, corn, tomato, lettuce, alfalfa, and leek).
- AMF symbiosis may enhance the osmotic adjustment in plants conferring the maintenance of higher leaf water status.
- The use of the composts has clearly promoted the growth of date palm and underlying crops tested. The combination of low doses of native composts and indigenous AMF significantly improved the growth of *P. dactylifera* and the crop species.
- The combination of *G. irregularis* and 10% OCOMWWG compost has no positive effect on the production of shoot dry matter of wheat and corn but increased substantially the root biomass than control plants.
- The application of the various combinations and biological treatments in field
  conditions resulted in significant differences than the control. The tripartite combination of AMF-PGPR-compost significantly increased crop yields in all crop
  species: leguminous, cereals, and vegetable crops. This efficacy depends on the
  plant, the nature and dose of the compost, and the mycorrhizal and bacterial
  isolates tested.
- The use of such effective organic and biological amendments could constitute a biotechnological tool to improve yield and plant adaptation to soil and environmental constraints.

In general, our study elucidated the positive impacts of biofertilizers composts-AMF-PGPR on the growth, yield, and development of date palms and cultures underlying with the adoption of innovative practices. The application of composts

and/or microorganisms could improve soil fertility, preserve water resources, respect the environment, and ensure the development of sustainable organic agriculture. The transfer of this technology in the open field will have a positive impact on the oasis environment by generating socioeconomic and environmental benefits such as improving farmers' incomes, reducing poverty, and preserving natural resources.

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