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Updating Ecology and Distribution of Wild Truffles in Morocco

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Abstract: The distribution and abundance of the various truffle species are influenced by the climate, soil, and vegetation conditions. The setting of these characteristics is necessary for the potential species' cultivation. Here, we describe the ecological characteristics of Moroccan truffles and desert truffles and their associations with host plants. We also determine the climate and soil parameters relating to the geographic distribution and fructification of truffles and desert truffles. In contrast to truffles, which are found in sub-humid environments, desert truffles are found in semi-arid and arid regions of Morocco. The dissemination of desert truffles in the Mamora forest and oriental regions of Morocco is typically linked to the presence of *Helianthemum* sp., whereas the existence of truffles (*Tuber* spp.) in the Middle Atlas depends on the subsistence of *Quercus ilex* and *Q. faginea*. The truffles' and desert truffles' fructification depends mainly on the precipitation frequency. *Terfezia arenaria* and *Tuber oligospermum*, the two major desert truffles of Mamora forest, require an annual rainfall of 435 mm on average in slightly acidic soil. While the oriental and Highland desert truffles, namely *Terfezia boudieri*, *T. claveryi*, and *Tirmania* spp., require an annual precipitation average of 123 to 267 mm and a high $CaCO_3$ content. Otherwise, there is *Tuber aestivum*, localized in humid regions with a rainfall rate of more than 650 mm, and found under calcareous soil rich in organic matter with the presence of potential host plants, such as oaks, cedars, and pines. Our findings open up the possibility of successful cultivation of truffles and desert truffles having an economic interest through understanding their ecological requirements in Morocco.

Keywords: truffles; desert truffles; soil properties; climate; vegetation; geographic distribution; Morocco

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1. Introduction

Morocco benefits from a privileged geographic location between Africa and Europe, being in the northwest of the African continent [1]. This geographical position provides Morocco with remarkable bioclimatic diversity, ranging from humid in the Rif, the Middle, and High Atlas (altitudes exceeding 1500–2000 m), to arid in South Morocco, through to sub-humid and semi-arid in the plains and foothills [1].

Hence, Morocco includes a variety of truffle and desert truffle species that require different edapho-climatic and ecological conditions. The truffles in Morocco that refer to the *Tuber* genus are harvested in humid or sub-humid areas, while the desert truffles colonize arid, semi-arid, and desert areas. The desert truffles belonging to *Terfezia*, *Tirmania*, *Picoa*, and *Delastria* prefer arid and semi-arid regions, either in sandy or loam soils, acid or calcareous soils, and arenaceous or light soils [2–6]. These various species are distinct from each other by their area of harvest, size, color, and host plant. Their main natural distribution is in the Mamora forest localized in the northwest, the plains of the Highlands in the east, and the Sahara in the south and southeast of Morocco [2,3]. The desert truffles in Morocco can establish symbioses with annual or perennial plants, especially those belonging to the Cistaceae family (*Helianthemum*, *Cistus*), while truffle species associate with woody plants [2,7–9].

These hypogeal fungi grow only from November to April, while others grow between March and May. The volume, as well as the quality, of the harvest varies according to rainfall and weather conditions [2,4]. Therefore, the truffles' distribution and abundance depend highly on the rainy seasons, amount of precipitation, climatic conditions, humidity, and types of soil [10,11]. The environmental parameters for the species could be different or sometimes similar based on the altitude, geographical location, the period of measurement and the season [12]. The main goal of the present study is to describe the natural habitat of Moroccan truffles and desert truffles and to identify the climate and soil characteristics of the collection sites that determine their geographic distribution and fructification.

2. Materials and Methods

2.1. The Study Area

The study area covered eight sites in Morocco, located in three natural habitats for truffles and desert truffles in Morocco (Figure 1). The first region was located in the Mamora forest to the east of Rabat city, which covers about 130,000 hectares. The second region was in the eastern regions of Morocco that starts at Ain Beni-Mathar, and extends to Tendrara, Bouarfa, and Figuig. The attitude of this area varied from 1000 to 2000 m. The third region was situated in the Middle Atlas, covering Azrou, Ifrane and Immozer-Kandar. The attitude of this area ranged from 1100 to 1600 m (Table 1). The choice of the collection sites was made through the investigation of truffle collectors and the presence of the host plants.

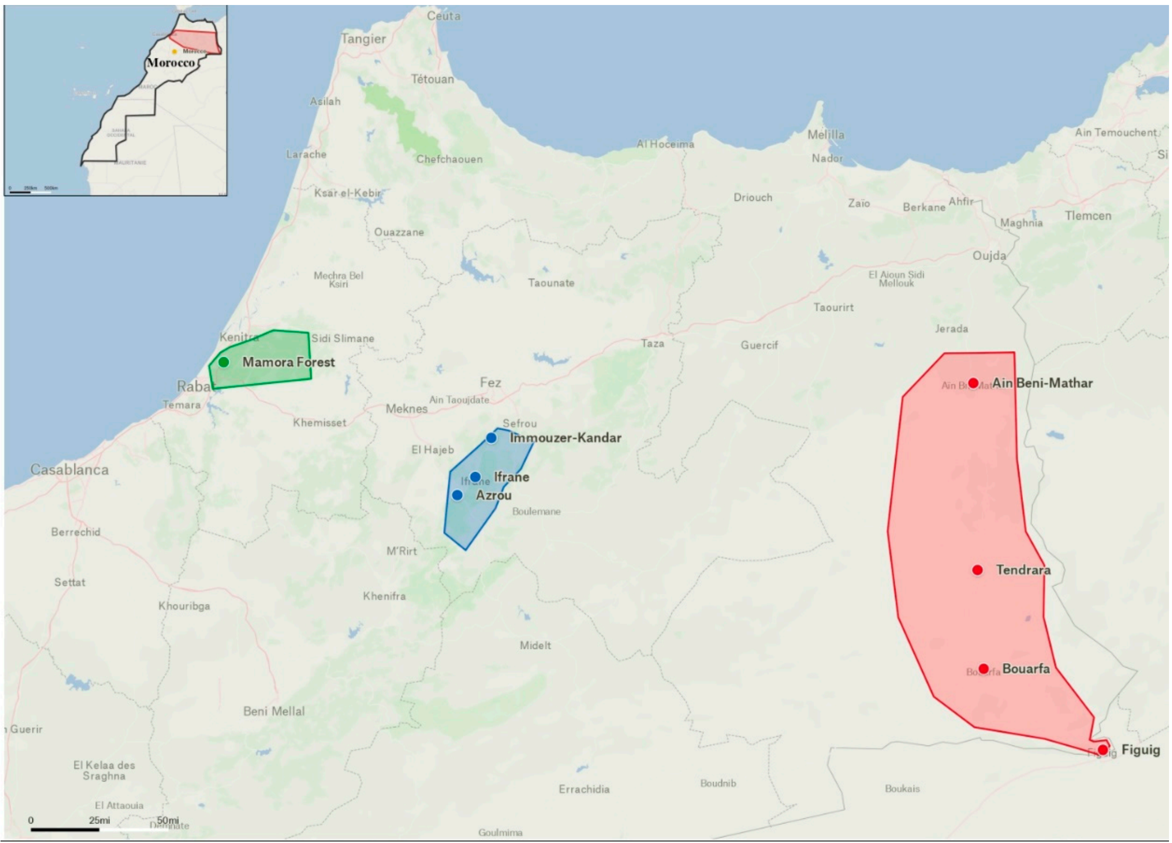

Figure 1. The location of eight potential sites where the natural truffles and desert truffles grow in Morocco.

Table 1. Altitude, GPS details, and Köppen–Geiger climate classification of the three wild truffle areas of Morocco.

Region	Site	Altitude (m)	GPS Localization	Climate Classification of Köppen–Geiger
Rabat	Mamora Forest	280	34°15′52″ N, 6°39′27″ W	Csa (Temperate)
Eastern Morocco	Ain Beni-Mathar	921	34°0′30.38″ N, 2°1′55.98″ W	BSk (Arid—Steppe)
	Tendrara	1451	33°2′60″ N, 2°0′0″ W	BSk (Arid—Steppe)
	Bouarfa	1174	32°31′51″ N, 1°57′47″ W	BWk (Arid—Desert cold)
	Figuig	900	32°7′29.00″ N, 1°13′33.89″ W	BWh (Arid—Desert hot)
Middle Atlas	Azrou	1250	33°26′29.21″ N, 5°13′29.27″ W	Csb (Temperate)
	Ifrane	1665	33°30′22.49″ N, 5°9′2.80″ W	Csb (Temperate)
	Immouzer-Kandar	1450	33°43′48.00″ N, 5°0′36.00″ W	Csb (Temperate)

2.2. Ecological Study of the Environment

For each sampling station, the execution of the surveys was conducted from 2014 to 2021, depending on the presence of the truffle species. The carpophore collection in the Mamora forest was established with the guidance of women collectors experienced in harvesting desert truffles in this area. Similarly, the selection of the studied area in eastern Morocco and carpophore harvest were performed with the assistance of Bedouin seasonal collectors living there, while the detection of *Tuber* spp. in the Middle Atlas was based on the presence of the host trees and the assistance of a trained dog. Three plots of about 100 m^2 were established for plant species determination and carpophore collection at each site, regardless of rainfall and environmental variations. Rainfall data (annual averages, minimums, and maximums) and temperature data (annual averages, minimums, and maximums) were harvested from the NASA Langley Research Center POWER Project website, called POWER Data Access Viewer (https://power.larc.nasa.gov/data-access-viewer/ (accessed on 1 October 2022)) by inserting the Geographic Coordinates (Latitude/Longitude) of the selected sites as the input to relate the absolute location of each site. The harvested data were used as a basis for studying truffle ecology. Soil samples from the three major areas where truffles grow extensively were analyzed to determine the optimal soil physicochemical properties for truffle and desert truffle growth. The soil samples consisted of 10 samples taken from a depth of about 0–30 cm from an area of 100 m^2. The soil samples were air-dried, homogenized, sieved (2 mm sieve-mesh), and conserved in the fridge. The soil sample analysis was performed in the laboratory of soil analysis at ARRAS, INRA Clermont-Ferrand, France. The plant species were recorded to describe the vegetation composition using visual estimation methods of Braun-Blanquet cover-abundance scales [12]. Periodic outings (6 times a year) were carried out to the sites to determine the various species including trees, shrubs, and herbaceous plants. Fungus identification was performed through macroscopic and microscopic attributes [13–15]. The distinction between *Tuber gennadii* and *Tuber asa* was established following Agnello and Kounas [16] description.

2.3. Statistical Analysis

A climograph (bar and line graph) of the monthly average precipitation and temperature from 1995 to 2021 was obtained using packages dplyr and ggplot2 in R Studio [17]. The principal component analysis was performed using FactoMineR and factoextra packages [18]. A one-way ANOVA was performed on the means of the soil properties to compare between the three bio-climatic truffle-growing areas [19]. The Tukey HSD test was used to accomplish the pairwise comparisons among means. The multcompLetters4() function from the multcompView package was used to indicate significant differences [20].

3. Results and Discussion

The natural habitat of truffles and desert truffles has always been the basis of truffle research. In Morocco, the wild truffles have very different ecological requirements, such as climatic conditions, host species, and levels of soil pH. These differences lead to a variations in the geographical distribution of these hypogeal fungi around the country. In this report, we studied the ecology of three naturally grown truffle areas in Morocco.

3.1. Floristic Composition

The truffle species and their potential host plants found in the collection sites of Morocco are shown in Table 2. In parallel, Figure 2 displayed the geographic distribution of the truffle and desert truffle species in those sites. In Mamora forest, the wild truffle fields were easily recognized due to the denuded aspect of the soil. *Terfezia arenaria* and *Tuber oligospermum* were the most frequently crossed during the survey in Mamora. The typical tree species of this forest were cork oak (*Quercus suber*), *Pinus pinaster* var. atlantica and *Pinus mamorensis*. *Quercus suber* was an endemic non-host tree of Mamora, as well as the newly reforested *Eucalyptus camaldulensis* tree. *Pinus pinaster* was the host plant of truffles, notably *Delastria rosea* and *Tuber oligospermum*. *Pinus Pinaster* var. atlantica was one of the species used for reforestation in this forest [21]. The presence of *Tuber oligospermum* depends highly on the existence of *Pinus* trees. Our results confirm the findings by Marjanović et al. [22], who proved as well that *Tuber oligospermum* is harvested under a non-native tree species for the area (*Pinus halepensis* Mill.), indicating that *Pinus* tree species are the principal host plants of *Tuber oligospermum*. Additionally, *Cistus* spp. represented the intermediate shrubby host plants of truffles in Mamora forest (Figure 3a), especially *Cistus monspenliensis* and *Cistus salviifolius*. Louro et al. [23] reported that the *Terfezia*–*Cistus* partnership exhibited a high percentage of mycorrhization and declared that the *Cistus* is a potential host plant for *Terfezia* species. The herbaceous stratum in Mamora forest was characterized mostly by the dominance of *Tuberaria guttata* and by bulbous plants (ex: *Urginea maritima*, *Asphodelus microcarpus*, and *A. aestivus*). *Tuberaria guttata* (Figure 3b) is the main host plant of *Terfezia arenaria*, *T. leptoderma*, and *Tuber gennadii*. In Algeria, *Terfezia arenaria* was identified in the Madagh forest, Stidia forest, and Kharouba forest, located in the northwest of Algeria where it is associated with the same host plant in the presence of similar conditions to the Mamora forest [24]. The most common method of harvesting in Mamora forest is by the "mark" produced by the fungi (Figure 3c) and the women depended on this criteria to detect the location of mature truffles (Figure 3d).

Table 2. Truffle and desert truffle species identified and quantity harvested in the three investigated areas of Morocco, together with their potential host plants and maturity period.

Site	Truffle Species (According to Macro and Microscopic Morphology)	Average Amount of Carpophores from 2014 to 2021 (kg/100 m^2)	Potential Host Plant	Maturity Period
Mamora	*Terfezia arenaria*	9.50 ± 1.11	*Tuberaria guttata*	March to May
	Terfezia leptoderma	1.63 ± 0.37	*Pinus pinaster* var. atlantica *Tuberaria guttata*	3rd week of February until May
	Delasria rosea	1.25 ± 0.39	*Pinus pinaster* var. atlantica	November to December
	Tuber gennadi	0.71 ± 0.19	*Tuberaria guttata*	End of February to April
	Tuber oligospermum	1.32 ± 0.54	*Pinus pinaster* var. atlantica	December until April
Eastern Morocco	*Terfezia claveryi*	10.79 ± 2.57	*Helianthemum lippii*	March to May
	Terfezia boudieri	10.04 ± 3.44	*Helianthemum scoparium*	March to May
	Picoa juniperi	2.97 ± 0.88	*Helianthemum apenninum*	End of February
	Tirmania pinoyi	48.17 ± 12.04	*Helianthemum ledifolium*	mi-December until end of March
	Tirmania nivea	51.83 ± 9.92		mi-December until end of March
Middle Atlas	*Tuber aestivum*	1.00 ± 0.30	*Quercus ilex* *Quercus faginea*	April until the end of May
	Tuber excavatum	0.02 ± 0.01		
	Tuber brumale	0.06 ± 0.02		
	Tuber rufum	0.03 ± 0.01		

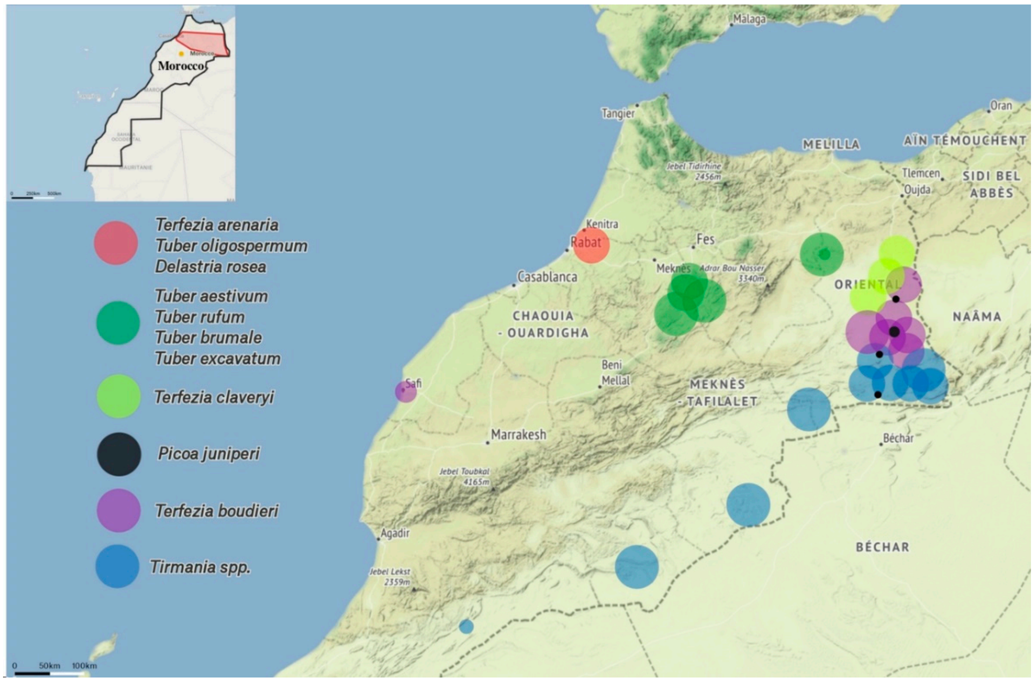

Figure 2. Geographic distribution of truffle and desert truffle species in studied sites in Morocco.

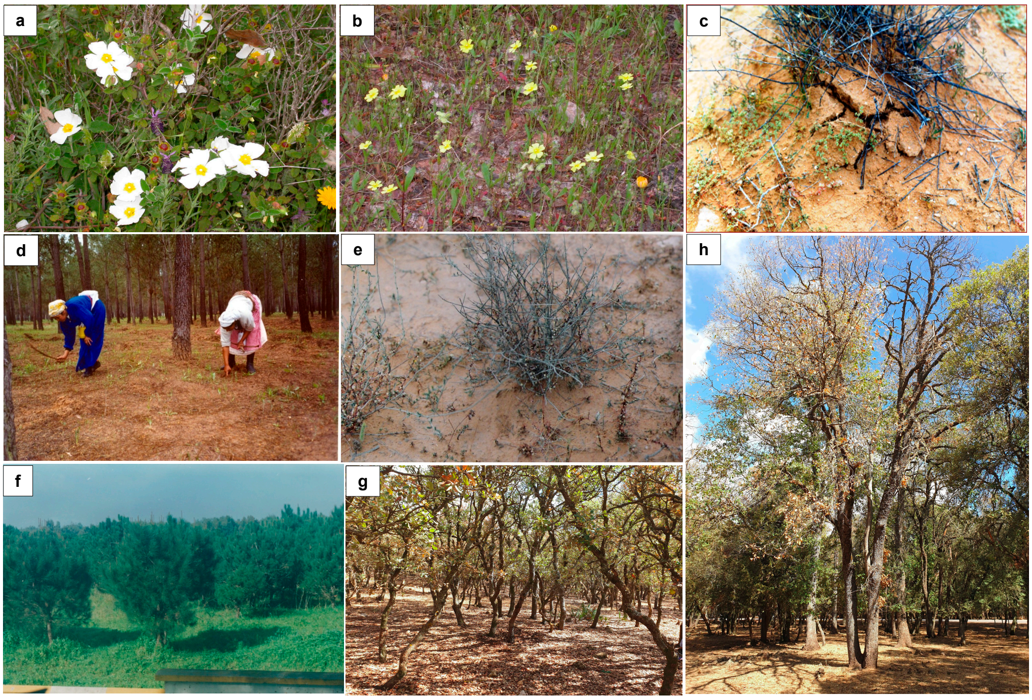

Figure 3. Host plant of truffles in Morocco. (**a**) *Cistus salvifolius* flowers in Mamora forest; (**b**) *Tuberaria guttata* in Mamora forest; (**c**) soil fissures indicate the presence of truffles under the host plant; (**d**) women look for truffles in Mamora forest; (**e**) *Helianthemum lippii* in the desert of Bouarfa; (**f**) *Pinus pinaster* tree in Azrou; (**g**) *Quercus ilex* in Jaaba forest of Ifrane; (**h**) *Quercus faginea* in Jaaba forest of Ifrane.

In the eastern highlands and southeastern Morocco, the vegetation is dominated by Alfa plants (*Stipa tenacissima*). This species occupies uniformly the west of the highlands, starting from Mlilla to Errachidia, where the Alfa grass finds its optimum bio-climatic conditions. In addition, *Stipa tenacissima* exists in the mountain hills of the region, especially in the east of Debdou, the western of Beni-Snassen Mountains, and the undulating reliefs that extend to El-Ayoun, Jerada, and the Moroccan–Algerian border. In the south, the Alfa gives way to distinctly Saharan species, such as *Aristida obtusa*, *Fredolia* woody steppe, and *Haloxylon scoparium*, a clear steppe more or less buried in sandy formations. Nevertheless, *Stipa tenacissima* is still present in some very advanced sectors between Figuig and Boudnib. The most frequent desert truffles species found in this area was *Tirmania pinoyi*, *Terfezia boudieri*, and *T. claveryi*, while *Picoa juniperi* was a very rare species found mainly in Tendrara. Those species established mycorrhization with *Helianthemum* spp., particularly *Helianthemum lippii* (Figure 3e), *H. ledifolium, and H. apenninum*, which confirms the results of Bermaki et al. [25]. *Terfezia*, *Picoa* and *Tirmania* were endemic to arid and semi-arid areas of the Mediterranean region [26], where they were associated with *Helianthemum* species. Morte et al. [5] added that the disposition of desert truffles depends on the presence of *Helianthemum* spp., since the desert truffles form mycorrhizal associations with these host plants. Therefore, the endurance of desert truffles is ensured by the presence and persistence of *Helianthemum* spp. in this region.

In the Middle Atlas, the natural habitat of black truffles (*Tuber* spp.) is divided into three main types. The sub-humid region in Immouzer-Kandar was dominated by *Quercus ilex*, *Hyphaene thebaica*, *Juniperus oxycedrus*, *Arbutus unedo*, and *Cistus villosus*. Meanwhile, in the humid forest of Ifrane, a mixture of *Quercus ilex*, *Q. faginea*, *Q. suber*, *Cedrus atlantica*, *Daphne laureola,* and *Crataegus oxyacantha* were identified. Plus, the Jaaba forest was highlighted by the presence of *Chamaerops humilis* alongside *Quercus ilex*. Otherwise, the Azrou region was dominated by *Pinus pinaster* (Figure 3f). The common *Tuber* species found in this region were *Tuber aestivum*, *T. rufum*, *T. brumale*, and *T. excavatum*. This finding was also reported by Stobbe et al. [27], who revealed that *T. rufum* developed in the same habitat as *T. excavatum* and *T. aestivum*. *Tuber* spp. of the Middle Atlas were detected particularly under *Q. ilex* (Figure 3g) and *Q. faginea* (Figure 3h). Garcia-Montero et al. [28] found that *Tuber aestivum* can establish mycorrhizal associations with various host plants, over 20 species, other than *Quercus* spp. [28]. Among them, the *Pinus* tree can be a potential host plant for *Tuber aestivum* production. Furthermore, Garcia-Montero et al. [28] reported that *Pinus* produces better carpophores of *Tuber aestivum* in natural conditions compared to *Q. ilex* and *Q. faginea*.

3.2. Climatic Data

The annual average of precipitation and temperature, the relative humidity and the bioclimatic classification of the eight sites are summarized in Table 3. The Mamora forest was characterized by a humid and temperate climate with hot and dry summers (Mediterranean climate). The average temperature in Mamora was about 25 °C in the warmest month and 15 °C in the coldest month (Figure 4), and the average annual precipitation is 435.73 mm. *Terfezia arenaria* in Mamora forest requires a maximum rainfall of 240 mm on average before March, while other *Terfezia* spp. require a rainfall amount of less than 200 mm for a good harvest (100 tons/year) [3]. A decrease in rainfall with a slight rise in temperature during the ripening period (March–May) is also necessary [3]. Adequate temperature values during this period are 14 to 18 °C [3]. The temperature is not the major determining factor: the fruiting of Mamora truffles depends mainly on well-distributed precipitation during the growth period (Figure 5). Bradai et al. [29] demonstrated that the abundance of *Terfezia arenaria* was positively related to the autumnal precipitations occurring from October to December that represent the germination season of this truffle along with the host-partner *Helianthemum*. They added that the abundance of this species was negatively correlated with minimal temperatures and precipitation from January to March. The precipitation frequency between October and March is essential for the *Terfezia* spp., as well as for the host plants, *Helianthemum* spp. Bradai et al. [29] affirmed that 180 mm of well-distributed precipitation from October to March increased the density of the host plant and increased their mycorrhization with desert truffles.

Table 3. Climatic data of the three natural habitats of truffles and desert truffles analyzed in this study.

Region	Site	Relative Humidity (%)	T Min (°C)	T Max (°C)	Annual Average Temperature (°C)	Annual Average Precipitation (mm)
Rabat	Mamora	74.16	7.33	37.31	18.85	435.73
Eastern Morocco	Ain Beni-Mathar	55.18	−4.16	40.50	16.07	266.89
	Tendrara	49.15	−4.85	39.17	15.65	160.74
	Bouarfa	45.50	−4.49	40.13	16.78	226.85
	Figuig	38.39	−3.08	42.74	19.43	123.54
Middle Atlas	Azrou Ifrane Immouzer-Kander	59.37	−5.22	38.18	14.38	658.87

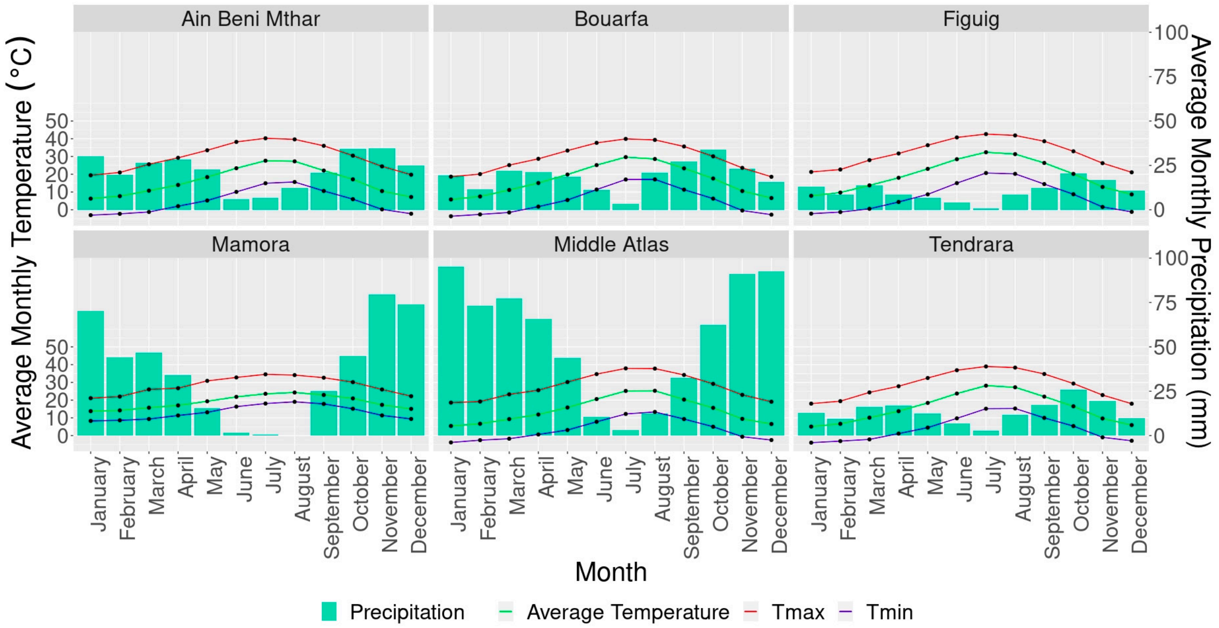

Figure 4. The average monthly temperature and precipitation of the studied sited in Morocco from 1995 to 2021.

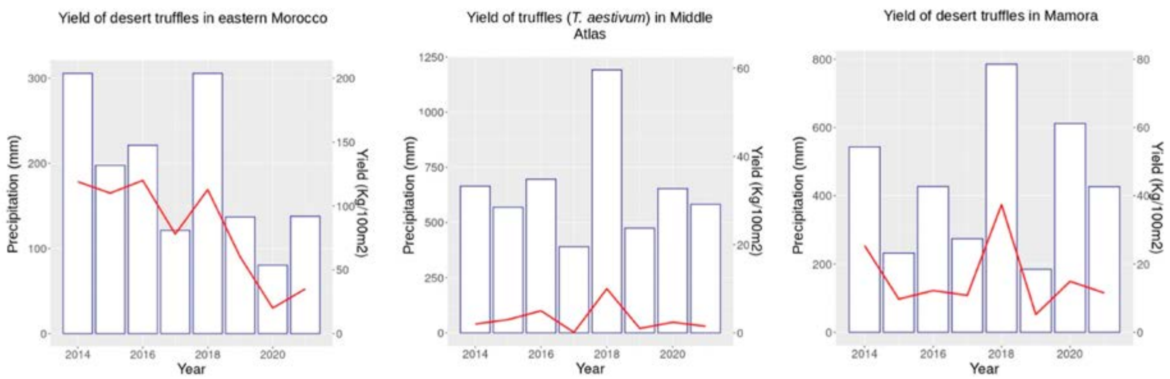

Figure 5. Graphs from 2014 to 2021 illustrating the variation in desert truffle and truffle yields (kg/100 m^2) in relation to the variance in precipitation (mm).

The oriental regions (Ain Beni-Mathar, Tendrara, Bouarfa, and Figuig) were characterized by an arid and Saharan–Mediterranean climate with an irregular rainfall rate ranging from 267 mm/year in Ain Beni-Mathar to 123 mm/year in Figuig (Table 3). The number of rainy days could be 30 to 40 per year [3]. The highest temperatures were around 37 °C and the lowest were around −4 °C. *Terfezia claveryi* was an abundant species in Ain Beni-Mathar, Tendrara and Bouarfa, in the presence of sufficient and regular precipitation in winter. Furthermore, *Tirmania* spp. were typical truffles of this region. Those sub-Saharan species could spread until Figuig and the south of Morocco. Otherwise, *Terfezia boudieri* was present in Ain Beni-Mathar and Bouarfa, where there was satisfactory and well-distributed precipitation during the fruiting period. Bermaki et al. [25] reported that *Tirmania* spp., *Terfezia claveryi*, and *Terfezia boudieri* were abundant in Figuig province. *Tirmania* spp. dominated Bni Guil, while *Terfezia* spp. occupied the Abou Lakhal region, and *Picoa juniperi* was identified only in Jebel Maiz. Bradai et al. [29] revealed that *Tirmania nivea* was the dominant species in the hyper-arid conditions of Algeria. In addition, they found that this species was highly productive, while *Terfezia claveryi* was more disrupted by the scarcity of the autumnal and annual precipitations. These results confirm the sensitivity of *Terfezia claveryi* and *Terfezia boudieri* to the climatic conditions and particularly to the lack of precipitation, restricting their presence in few regions compared to *Tirmania* spp., which could resist and disseminate in the hyper-arid areas. An optimal desert truffle production necessitates at least 100 mm of rainfall (Figure 5). Tadja [30] reported that a good production of wild truffles needs a rainfall amount ranging from 40 to 60 mm between October and November [30], followed by 26 mm of rainfall in March [31]. Nevertheless, no production will be obtained if the precipitation does not exceed 51 mm [29].

The climatic condition of the Middle Atlas (Imouzzer-Kander, Ifrane, and Azrou) according to the Köppen classification is Csb, with a humid and temperate climate and a dry summer (Mediterranean climate). The average temperature of the warmest month was 20 °C, while that of the coldest month was 5 °C (Figure 4). The average annual precipitation in this area reached 659 mm. It reached 40 mm in May and exceeded 70 mm in November, December, and January. *Tuber aestivum*, as one of the wild *Tuber* species found in this region, preferred a high rainfall rate in the winter (90 to 100 mm in December and January) and a moderate rainfall in summer (12 mm in August). Despite this, *T. aestivum* productivity is typically low and rises proportionally to increases in precipitation levels (Figure 5). The mean annual precipitation in several studied environments of *Tuber aestivum* was 825 mm, with generally more precipitation in summer (between May and August). In addition, the mean temperatures fluctuated from −0.7 °C in January to 17.4 °C in July with a yearly average of 8.25 °C [27]. Fischer et al. [32] mentioned that the *Tuber* spp. prefer areas that do not demonstrate severe or extreme temperatures. The temperature of the winter is particularly crucial because it influences the truffle maturation and production. Excessively low temperatures (−10 °C) for more than five days are harmful for truffle repining. Based on these outcomes, the studied sites of the Middle Atlas are suitable for the cultivation of *Tuber aestivum* due to the presence of an optimum climatic requirement for the species. Our findings confirm the conclusion of Khabar [4], who reported that the massive Debdou, the High Atlas and the Rif mountains of central Morocco are potentially adequate for *Tuber* spp. cultivation due to their humid and subhumid climates and generally calcareous soils.

3.3. Soil Analysis

The physicochemical properties of the soil in the natural habitats of wild desert truffles and *Tuber* spp. are summarized in Table 4. A notable variation was found between the three areas, indicating that the soil attributes and their proportions can differ from one geographical location to another. The soils of Mamora were slightly acidic (pH = 5.93) with a high proportion of sand (86%), which indicate a low water retention, with high permeability and better aeration. Nevertheless, the amounts of available nutrients and organic matter were low. This event could be explained by the low inputs of organic matter into the soil and the fast mineralization of organic components, since the availability of phosphorus in soil is low (58.77 mg kg^{-1}). Bonifacio et al. [33] reported that the soils have low productivity when their phosphorus contents oscillate from 32 to 273 mg kg^{-1}. Nevertheless, the phosphorus-deficient soil boosts the desert truffles' mycorrhization in soils.

Table 4. Soil characteristics from the greatest truffle growing areas (Mamora, Ifrane, and Bouarfa).

Component	Mamora	Ifrane	Bouarfa
pH	5.933 ± 0.152 [c]	7.866 ± 0.351 [b]	8.740 ± 0.180 [a]
Clay %	10.276 ± 0.116 [b]	32.466 ± 1.234 [a]	26.0 ± 1.732 [a]
Loam %	3.810 ± 1.223 [c]	45.100 ± 2.306 [a]	15.333 ± 5.033 [b]
Sand %	86.013 ± 1.062 [a]	22.433 ± 1.365 [c]	58.667 ± 3.512 [b]
Organic matter %	1.110 ± 0.223 [b]	8.303 ± 4.481 [a]	0.943 ± 0.159 [b]
Carbon %	0.643 ± 0.132 [b]	4.603 ± 2.689 [a]	0.686 ± 0.127 [b]
Nitrogen %	0.082 ± 0.027 [b]	0.382 ± 0.225 [a]	0.0717 ± 0.017 [b]
C/N	8.080 ± 1.355 [b]	12.067 ± 0.208 [a]	9.653 ± 0.498 [b]
$CaCO_3$ g kg^{-1}	0.109 ± 0.003 [c]	380.0 ± 14.731 [b]	1044.1 ± 55.761 [a]
P_2O_5 availability mg kg^{-1}	58.766 ± 10.813 [a]	80.333 ± 1.527 [a]	15.587 ± 4.783 [b]
CaO mg kg^{-1}	569.0 ± 12.124 [c]	12,377.0 ± 1011.660 [a]	10,700.0 ± 317.350 [b]
MgO mg kg^{-1}	75.313 ± 32.796 [c]	1480.0 ± 16.643 [a]	302.056 ± 60.776 [b]
K_2O mg kg^{-1}	26.586 ± 2.707 [b]	578.333 ± 11.504 [a]	505.853 ± 132.010 [a]
Exchangeable Ca mg kg^{-1}	317.280 ± 62.548 [c]	8760.0 ± 209.702 [a]	7680.0 ± 478.434 [b]
Exchangeable Mg mg kg^{-1}	45.370 ± 19.757 [c]	891.0 ± 10.583 [a]	181.837 ± 36.586 [b]
Exchangeable K mg kg^{-1}	22.156 ± 2.257 [b]	479.0 ± 16.522 [a]	419.857 ± 109.567 [a]

Data shown as mean ± standard deviation (n = 3). Different superscript letters in the same raw data indicate a statistically significant difference between sites ($p < 0.05$).

In the Ifrane soil where *Tuber* spp. grow, the clay and loam contents were around 32 and 45%, respectively, with a reasonable percentage of sand (22%). The pH was 7.86 (alkaline soil), while the organic matter was relatively abundant, well decomposed, and stabilized (C/N = 12). The calcium carbonate ($CaCO_3$) content was often high. The abundance of exchangeable calcium was 20 times more than Mamora soil. This abundance was linked to the richness of the soil in carbonates. Rainwater saturated with carbon dioxide could increase the solubility of $CaCO_3$ and convert calcium to an exchangeable form [33]. Similarly, in Bouarfa (oriental region), the pH was alkaline (pH = 8.74) and the calcium carbonate content was significant, which explains the high concentration of exchangeable CaO in the soil (Table 4). It was reported that the genus *Tirmania* was more extensive in soils with high $CaCO_3$ and silt percentages than *T. claveryi* and *P. juniperi* [26], which explains the abundance of *Tirmania* in this region. However, due to the desert environment and the scarcity of the plant community, very little organic matter was registered, which explains the low availability of phosphorus in the soil. Principal component analysis results (Figure 6) between the physicochemical characteristics of the soil and the production of truffles explained 99.7% of the total variation (PC1: 28.3% and PC2: 71.4%). The high organic matter, Mgo, Mg, carbon percentage, and nitrogen percentage were correlated with *T. aestivum*, *T. rufum*, *T. brumale*, and *T. excavatum* production. Additionally, the $CaCO_3$ and pH levels were correlated with *T. nivea*, *T. pinoyi*, *T. boudieri*, and *T. claveryi* production. Otherwise, there was no association between the Mamora truffles and any physicochemical element, showing that the optimal conditions for *T. arenaria*, *T. oligospermum*, and *T. leptoderma* are are low pH, low $CaCO_3$, and low organic matter. The pH and carbonate abundance in the soil controls the presence of truffle species and fruit-body richness in situ. *Terfezia arenaria*, *T. leptoderma*, and *Tuber oligospermum* are acidophilic species [5,34]. These species grow mainly in Mamora forest, where the soil is acidic, whereas *T. claveryi* and *T. boudieri* thrive in alkaline environments such as Spain [5] and were the most abundant species in eastern regions of Morocco as well.

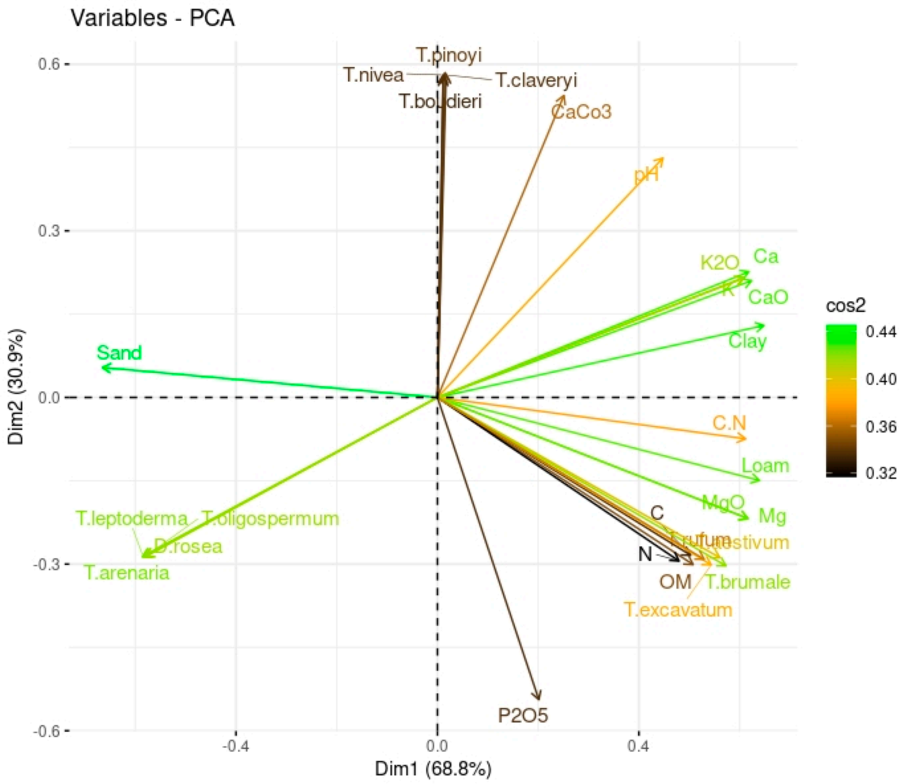

Figure 6. Principal component analysis (PCA) linking Moroccan truffle production to the physicochemical characteristics of the soil. pH: pH, Clay: Clay %, Loam: Loam %, Sand: Sand %, OM: Organic matter %, C: Carbon %, N: Nitrogen %, C.N: C/N, CaCo3: $CaCO_3$ (g^{-1} kg^{-1}), P2O5: P_2O_5 availability (mg kg^{-1}), CaO: CaO (mg kg^{-1}), MgO: MgO (mg kg^{-1}), K2O: K_2O (mg kg^{-1}), Ca: Exchangeable Ca (mg kg^{-1}), Mg: Exchangeable Mg (mg kg^{-1}), K: Exchangeable K (mg kg^{-1}).

4. Conclusions

The environmental conditions of the Mamora forest, oriental regions, and Middle Atlas are very favorable for the presence of truffles or desert truffles. The reforestation of Mamora forest with *Pinus pinaster* will improve the yield of *Tuber oligospermum*, which does not exceed 30 tons per year since it is well appreciated by the citizens and the tourists. In oriental regions, a well-distributed precipitation promotes the production of *Terfezia claveryi*, *T. boudieri* and *Tirmania* spp. from 400 to 1000 tons per year, while in the Middle Atlas regions, the annual production and commercialization of *Tuber aestivum* are still unknown due to the difficulty of harvesting in the wild. The Middle Atlas presents positive indicators for establishing future plantations using *Tuber aestivum* mycorrhized seedlings. Thus, following the example of *Tuber melanosporum*, which is not a native species of Morocco and was not identified during the survey, it would be actually and successfully cultivated in a plantation of 2.5 ha in Debdou and Imouzzer-Kandar, localized in the Middle Atlas, with an annual production of 30 kg using mycorrhized seedlings with *T. melanosporum* originating from France.

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