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Microbiological and Physico-Chemical Characterization of Soil of an Agroforestry System in Tetela De Ocampo, Puebla México

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The loss of the physicochemical and microbiological properties of soils, it is linked to the use of conventional agricultural practices. Knowledge of partnerships within agroforestry systems helps to establish management practices in order to restore and conserve the environment, preserving their physical, chemical and microbiological characteristics. This investigation aims microbiological identification and physicochemical determination of the soil present in the vegetation an agroforestry system in Tetela de Ocampo, Puebla-México. Was confirmed the presence of 14 plant species, 3 species of grasses are reported with forage use and 11 herbaceous two of which are legumes with forage use. The soil is classified as loam clay, with a Dap of 1-1.3 g cm^{-3}, with an acid pH, influencing the establishment of certain microbial groups adapted to these environmental conditions. The content of organic matter was predominant in medium scale, the values of total N, P and K were variable in all sampling points. Besides the presence of *Pseudomonas*, *Coliform* spp., *Trichoderma* spp., *Penicillium* sp., *Fusarium* spp., *Pythium* spp., *Aspergillus* spp., and *Rizophus* sp. was identified.

Keywords: Soil, characterization, diversification and agroforestry.

The use of agricultural and livestock conventional practices absent of planning and proper management directly affect the physicochemical and microbiological properties of soils; among which stand out, the change in land use, excessive use of pesticides, synthetic fertilizers, besides extensive livestock [1,2].

The inadequate grassland management and overgrazing favor the loss of vegetation cover and increase susceptibility to erosion as well as soil compaction, decline in organic matter, decreased nutrient and beneficial microorganisms [3-6].

Agroforestry systems are characterized by being highly diversified and self-sufficient [7], the interrelated components makes its heterogeneity more complex and provides the necessary conditions for maintaining edaphic micro-biota[8]. Besides biological interactions benefit fundamental ecosystem processes of these systems, providing an improvement in the physical, chemical and microbiological properties of soils [4-10].

The knowledge of partnerships within diversified systems would help to establish management practices with the goal of increasing synergies and at the same time preserve the biodiversity of agroforestry systems well as simultaneously promote the protection of soil erosion[11,12]. The physical, chemical and microbiological features help to characterize and determine soil quality and its potential in forest plantations, for that reason, the objective of this study was the microbiological identification and physicochemical determination of soil presents in

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vegetation of an agroforestry system in Tetela de Ocampo, Puebla-México.

MATERIALS AND METHODS

Study Site

The agroecosystem characterization comprising the physicochemical and microbiological valuation of soil, as well as the identification of native species of grasses and herbs present in the agroforestry system to detect forage use potential[13]. The system studied has a surface of 1.88 ha and is located in the municipality of Tetela de Ocampo, Puebla-México, under the coordinates 19º 49 '0.1' 'N; 97º 47 '33' 'O, to 1790 masl. Sampling was performed in early 2015, where 5 sampling areas were placed under the recommendations of the NOM-021SEMARNAT[14].

Soil physicochemical analysis

The physicochemical analysis of soil fertility was conducted in the department of Edaphology at the Institute of Sciences of the Autonomous University of Puebla (BUAP) to determine the most important features, such as the determination of pH using a potentiometer on a suspension of soil and water at a ratio of 1:2; electrical conductivity (dS m^{-1}), bulk density (g cm^{-3}), the % organic matter by the method of Walkley and Black, the % total nitrogen by the method of micro-Kjeldah, texture by the process of Bouyoucos to be identified in the texture triangle , extractable phosphorus (mg kg^{-1}) by the method of Bray and Kurtz, the cation exchange capacity using ammonium acetate 1N at pH 7 and the determination of exchangeable bases C mol kg^{-1} Calcium, Magnesium, Potassium, with ammonium acetate 1N at pH 7.

Characterization and identification of microorganisms

Soil microbiological analysis corresponds to counting the number of viable cells in solid medium for both fungi and bacteria. Saubouraud broth and nutritive agar were used for bacteria; afterward a series of serialized dissolutions was performed based on 10 colony forming units (CFU) for planting a specific volume in solid medium and obtain axenic cultures for later identification[15]. Identification of bacteria was performed using the Compact Dry kit "Nissui" EC following the directions according to the manufacturer, it was incubated for 24 h at 37 ° C to finally see the growth denoted by the color of the colony on culture plates[16].

In the case of fungi, one gram of soil was inoculated on PDA medium and incubated at room temperature for 3 to 5 days, the different fungal colonies that were observed were isolated individually by reseeding to obtain axenic cultures, with different macroscopic characteristics. The identification was made through taxonomic keys based on the color of the colony, their morphology, method of production and management of spores, is also important to know the size and disposition of the hyphae[17,18 and 19].

The statistical program SPSS Statistics version 17 (Statistical Package for the Social Sciences) was used, the data obtained were processed using the analysis of variance (ANOVA) and then the Tukey test of multiple comparisons (á = 0.05) was applied to determine the differences among study agro-ecosystem sites.

RESULTS AND DISCUSSION

Plant diversity

The topological arrangement of agroforestry plantation is in real framework, having a spacing of 2.5 m, between columns and rows of *Pinus greggii* and five years of planting for the establishment of a seed orchard, due to its importance in the sawmill industry in México, adding to the conversion of agricultural land to forest plantations in the American continent in the recent decades[12].

Was confirmed the presence of 14 outstanding agroforestry plant species, among which, 3 species of grasses are reported with forage use and 11 herbaceous two of which are legumes with forage use. The use of herbs and pastures in silvopastoral systems can be optimized through the selection of species and fertilization[20]. The site with more vegetative abundance is the site B, featuring 10 species, 3 grasses and 7 herbaceous. The site with a lower abundance was the C, all sampled species have a high forage potential[21,22 and 29]. The pastures predominated in all sampling sites, the percentages of abundance are shown in Tables 1 and 2.

When comparing the overall averages of physical soil properties, significant differences

Table 1. Characterization of pastures for agroforestry system site in Tetela de Ocampo, Puebla-México

Site	Pastures	Forage Potential	Abundance %
Site A	*Cenchrus clandestinus* (Hochst. ex Chiov)	High [21]	42.85b
	Bothriochloa laguroides (DC) Herter	High [22]	
	Eragrostis sp	High [23]	
Site B	*Bothriochloa laguroides* (DC)	High [22]	30.00d
	Cenchrus clandestinus (Hochst. ex Chiov)	High [21]	
	Eragrostis sp	High [23]	
Site C	*Bothriochloa laguroides* (DC)	High [22]	60.00a
	Cenchrus clandestinus (Hochst. ex Chiov)	High [21]	
	Eragrostis sp	High [23]	
Site D	*Bothriochloa laguroides* (DC)	High [22]	42.85b
	Cenchrus clandestinus (Hochst. ex Chiov)	High [21]	
	Eragrostis sp	High [23]	
Site E	*Bothriochloa laguroides* (DC)	High [22]	33.33c
	Cenchrus clandestinus (Hochst. ex Chiov)	High [21]	
	Eragrostis sp	High [23]	

*Means with different letters in the column indicate significant differences with the Tukey test (á ≤ 0.05), for sampled area

Table 2. Characterization of herbaceous for agroforestry system site in Tetela de Ocampo, Puebla-México.

Site	Herbaceous	Forage Potential	Abundance %
Site A	*Bidens odorata* Cav. Var. *Odorata*	Low [24]	57.14c
	Fleischmannia pycnocephala (Less)	Low [25]	
	Medicago polymorpha L.	High [26]	
	Oenothera rosea L'-Hér. Ex Aiton	Low [27]	
Site B	*Crotalaria* sp	Low	70.00a
	Erigeron karvinskianus DC.	Low [28]	
	Oenothera rosea L'-Hér. Ex Aiton	Low [27]	
	Oenothera tetraptera Cav.	Low	
	Plantago lanceolata L.	High [29]	
	Pseudognaphalium sp.	Low	
	Trifolium repens L.	High	
Site C	*Medicago polymorpha* L.	High [26]	40.00d
	Plantago lanceolata L.	High [29]	
Site D	*Bidens odorata* Cav. Var. *Odorata*	Low [24]	57.14c
	Medicago polymorpha L.	High [26]	
	Oenothera rosea L'-Hér. Ex Aiton	Low [27]	
	Plantago lanceolata L.	High [29]	
Site E	*Bidens odorata* Cav. Var. *Odorata*	Low [24]	66.66b
	Medicago polymorpha L.	High [26]	
	Monnina xalapensis Kunth	Low [30]	
	Oenothera rosea L'-Hér. Ex Aiton	Low [27]	
	Plantago lanceolata L.	High [29]	
	Pseudognaphalium sp.	Low	

*Means with different letters in the column indicate significant differences with the Tukey test (á ≤ 0.05), for sampled area.

were found among the five sites within the agroforestry system, texture regarding a relationship with the bulk density is defined, data for most of the sites are placed on the scale of loam soil since they enter the range of 1-1.3 g cm^{-3} Dap, Murray[31] reported an improvement in soil bulk density of an agroforestry system at 5 years of establishment with 1.19 g cm^{-3}. Also the textures are considered average, as well defined by their percentages of sand, silt and clay (Table 3), the site C differs by having a Dap value of less than 1, which ranks it as soil derived from volcanic ash, however, it is more common to find this kind of soil with average texture, so is considered a homogeneous texture at all sampling points. Texture plays an important role in the ability to regenerate the function and eco systemic services in degraded soils, likewise clay content affects microbial habitat also to influence the formation of aggregates which in turn affect the diversity of soil habitat, suggesting a strong interaction between the soil structure and the recovery of biomass of different microbial groups[3, 2].

The bulk density values consider them loam soils, Ndaw[33] mentions that densities within this classification are considered favorable for agriculture, since, for its texture have good moisture retention and reduced mechanical resistance in favor of radical penetration.

The pH is an important measure in the analysis of soil since it controls chemical and biological reactions[6, 34]. The range on the five sampling sites goes from moderately acid to acid for the site C, reaching a neutral pH for Site E (Table 4), this way the acid scale predominated in most sampled points coinciding with Ndaw[33] where acid pH values are reported in soils of forest and silvopastoral systems, Rigueiro[35] mentions that during the establishment phase of diversified systems for a period of about 5 to 8 years, soils recorded acidic pH values, similarly tree species participate in the quantity and composition of organic material into transformation process, in this sense the needles of *Pinus greggii* are acidic and are in continuous deposition on the ground, another factor that can influence the decrease in pH, promoting the establishment of certain microbial groups, which would be adapted to the environmental conditions imposed by management practices of agroforestry systems[6].

The content of organic matter (MO) goes based on soil texture, in most points (site A, B and D) are considered high ranking unlike sites (C and E) standing at the average level in MO content.

Table 3. Determination of soil physical properties from the agroforestry system

Site	Texture	Sand %	Loam %	Clay %	Dap g cm^{-3}
Site A	Loam clay	46a	30a	24a	1.30a
Site B	Loam clay	42a	33a	25a	1.30a
Site C	Clay	40a	34a	26a	0.04c
Site D	Loam clay	33a	32a	35a	1.21b
Site E	Loam clay	30a	41a	29a	1.28ab

*Means with different letters in the column indicate significant differences with the Tukey test (á d"0.05), for sampled area.

Table 4. Determination of soil chemical properties from the agroforestry system

Site	pH	CE dS m^{-1}	MO %	N total %	P extractable %	K %	CIC Meq 100 mg^{-1}
Site A	5.6b	0.32a	1.76c	0.089c	23b	1.74c	19d
Site B	5.6b	0.33a	2.02b	0.101b	18e	2.63a	16e
Site C	5.4b	0.25b	0.75d	0.040d	19d	1.41d	22c
Site D	5.6b	0.30a	2.27a	0.111a	22c	1.89b	27a
Site E	7.0a	0.31a	0.60e	0.032e	28a	1.88b	24b

MO = Organic matter, CE = Electrical conductivity, N = nitrogen, P = phosphorus, CIC = Cation Exchange Capacity, K = potassium. *Means with different letters in the column indicate significant differences with the Tukey test (á d"0.05), for sampled area.

The parameter of electrical conductivity (CE) has at all sites values below 1 dS m^{-1} (Table 4), i.e., there is no problem of salinity or are free of salts according to the Official Mexican Standard[14].

The presence of N is certainly imperative for the development of agroforestry systems, followed by phosphorus and potassium[36]. The N results show total proximity with Rosas[37], with a total N content of 0.138% in an agroforestry soil is reported. For P and K the percentages show variability with a range from 18-28% for P an 1.41-2.63% for K, Suarez[38] obtained variable data for soils in agroforestry systems cultivated with coffee, 12.55-57.87% for P and 0.56-1.57% K.

Microbiological analysis

Microbiological conditions in the agroforestry system depends heavily on the physico-chemical soil conditions, the factors that influence the microbial metabolism are pH, temperature, oxygen and moisture, which in turn depend on the organic material[6]. Knowing the microbiological diversity is an important indicator of soil quality, because microorganisms are the basis of the food chain and are directly linked to nutrient cycling[33]. The number of bacteria and fungi identified among the five sites within the agroforestry system are shown in Table 5.

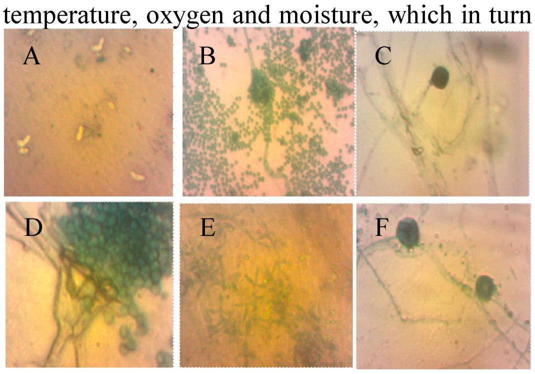

Fig. 1. Optical microscopy (40x) isolated fungal genera: A) *Fusarium*, B) *Aspergillus*, C) *Phytium*, D) *Penicillium*, E) *Trichoderma*, F) *Rizophus*

Through the microbiological analysis it was established 24 isolates, including two bacterial genera and six fungal genera associated with the agroforestry system, which were *Pseudomonas* y *Coliformes* spp., *Trichoderma* spp., *Penicilium* sp., *Fusarium* spp., *Phytium* spp., *Aspergillus* spp., y *Rizophus* sp.

Surely can be other bacterial genera present, however, the conditions that were submitted for identification did not allow their expression, just as only a fraction of the population is captured in specific time and space restricting a significant number of taxonomic units[39].

The site that showed higher values of UFC ml-[1] was the site A, likewise showed more diversity in fungal genera, with *Peniculium, Pythium, Aspergillus, Rizophus*. For sites C and D fewest isolates were obtained from which the genus *Trichoderma* feature being isolated in four sampling sites. Isolation of *Trichoderma* in cultivated soils relates to a wide adaptability to pH conditions, exhibiting outstanding antagonistic activity toward pathogens[40]. Most of the phenomena occurring in ecosystems are generally positive in terms of the interactions to the development of crops, both agricultural and forestry[41].

Table 5. Microbial population associated with the soil from the agroforestry system.

Site	Bacteria	Density %	UFC mL^{-1}	Fungi	Density %	UFC mL^{-1}
Site A	*Coliformes*	14.28	43.0 x 10^5 a	*Aspergillus*	14.28	14.6 x 10^3 a
	Pseudomonas	14.28		*Penicillium*	28.56	
				Phytium	14.28	
				Rizophus	14.28	
Site B	*Pseudomonas*	50.00	11.0 x 10^5 c	*Trichoderma*	50.00	8.0 x 10^3 b
Site C	*Coliformes*	33.33	31.2 x 10^5 b	*Trichoderma*	33.33	6.6 x 10^3 bc
	Pseudomonas	33.33				
Site D	*Pseudomonas*	50.00	29.0 x 10^5 b	*Trichoderma*	50.00	4.0 x 10^3 d
Site E	*Coliformes*	16.66	1.0 x 10^7 d	*Fusarium*	16.66	5.3 x 10^3 cd
	Pseudomonas	16.66		*Penicilium*	33.32	
				Trichoderma	16.66	

*Means with different letters in the column indicate significant differences with the Tukey test (α = 0.05), for sampled area

The family *Pseudomonadaceae* is a versatile genus, *Pseudomonas* spp., are present in pest control in agriculture, since some strains have the ability to act as antagonists towards phytopathogens by the production of antibiotics and siderophores, also, are considered promoting bacteria of plant growth[42]. Quagliotto[43] also study the association between a strain of *Pseudomonas* and *Medycago sativa*, showing increase in the rate of nodulation and dry matter of the plant.

The genera *Penicillium, Trichoderma, Fusarium, Aspergillus*, are considered among the most isolated and characterized in agriculture, forestry and in route of degradation soils[44]. The figure 1 shows some micrographs optical of the morphology of the fungi most commonly isolated.

The genus *Trichoderma* is known for reduce the severity of plant diseases through several mechanisms highlighting the role of parasitism[45]. The presence of *Fusarium* is correlated with abiotic factors as levels of clay, the ammonium concentration and organic matter, however the structure of their communities correlates with the microbial community in soil[46]. *Aspergillus* sp, has been linked to forest soils, Yang[47] have studied a strain able to degrade lignin, contributing to the search for more ecological processes in the paper industry, It has also been studied as degrader of agricultural soils contaminated with endosulfan, becoming an alternative to soil pollution problems and even water bodies[48,49].

CONCLUSION

The importance in plant, physicochemical and microbiological characterization lies with the priority of the current status of the agroecosystem, providing useful information to propose an agrosilvopastil management tending to sustainability. A greater diversity of herbaceous present in the agroforestry system was found; resulting two forage species of important character such as *Trifolium repens L.* and *Plantago lanceolata L.* representing a wealth of 70% in the agricultural ecosystem. However, the dominant grass species occupied more space, highlighting its availability throughout the year and high forage potential of *Eragrostis sp* and *Cenchrus clandestinus.*

The establishment of the agroforestry system is in clay loam soils with Dap of 1-1.3 g cm^{-3} and acid pH in most of the property, presenting a lower electrical conductivity than 1 dS m^{-1}, with plenty of organic matter and good soil fertility under the NOM-021 SEMARNAT. The agroforestry system has two bacterial genera and six fungal genera associated with the physicochemical soil conditions. *Pseudomonas* sp., showed higher abundance in soils with high content in organic matter and CE 0.33 and 0.30 dS m-1, however, the fungal diversity was affected by the neutral pH of soil, having more abundance in acid soils. Furthermore the fungal genera isolated such as *Trichoderma* spp., and *Penicillium* sp., possess biotechnological qualities potential for other research projects.

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