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Impact of Previous Vegetation Cover on Mycorrhizal Colonization and Performance of *Moringa oleifera* in Rainforest Regions of Cameroon

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Abstract: *Moringa oleifera* is a nutritional and medicinal plant. Conditions required for its cultivation have not yet been fully determined. This study was carried out in two localities in Cameroon to assess the impact of the previous vegetation cover (forest, *Chromolaena odorata* fallows and crop field) on mycorrhization and plant growth of *M. oleifera*. *M. oleifera* seedlings were grown in a greenhouse for 3 months in soil samples from the top soil layer. Plant height was measured every 2 weeks after sowing. Plant mortality, mycorrhizal colonization rate, plant height and biomass production were recorded after three months at the end of the experiment. Statistical analyses showed an effect of the type of previous land use on mycorrhizal colonization and growth of *M. oleifera*. The soil that had been *C. odorata* fallows was found to be more suitable for *M. oleifera* cropping in rainforest areas. In the second phase, soil biological and physicochemical properties will be determined to understand the extent to which these factors exert an impact on the mycorrhizal colonization rate and on *M. oleifera* performance.

Keywords: Moringa oleifera, mycorrhization, growth, previous vegetation cover, rainforest region, Cameroon

1. Introduction

Especially in underdeveloped countries where a malnutrition rate of over 80% prevails, the food deficit could be reduced by increasing the consumption of potentially nutritious plant species (FAO, 2002). *Moringa oleifera*, commonly called *Moringa* and drumstick tree, is known worldwide for its food, medicinal, oil and water-purifying qualities (Audru, 1988). As leaves, roots and seeds of the *Moringa* are edible; it could be used to combat malnutrition and associated diseases (De Saint Sauveur & Broin, 2006).

M. oleifera is a perennial tree species that can grow to a height of 10 m. The length of its compound leaves ranges from 30 to 70 cm. Its fruit pods reach maturity 5–6 months after flowering. Mature pods release round black seeds with two cotyledons, each having three lateral wings (Figure 1).

Figure 1. *Moringa oleifera* tree with flowers and immature pod

Analysis of the composition of *M. oleifera* leaves and fruits indicates that they contain essential amino acids, including arginine, histidine, lysine, phenylalanine, methionine, threonine, leucine, isoleucine and valine, as well as minerals and fibre (Foidl, et al., 2001). Its dried crushed roots are eaten as a condiment (Jumelle, 1930) whiles its leaves and young shoots are used to prepare soups (Busson, 1965). In several African and Asian countries, food for babies and pregnant women is often supplemented with *Moringa* leaf powder. Its fruits are consumed as a green vegetable and high quality cooking oil is extracted from the seeds. According to Dalziel (1955), *M. oleifera* roots and bark are used to remedy inflammation and joint pain. Its fruits are used to enhance sperm quality and quantity and also to relieve nervous weakness. *Moringa* seed powder can purify water via its flocculation properties, even neutralizing mobile germs (Audru, 1988), and it serves as a substitute for activated alum for water treatment. Moreover, the high quality oil obtained from *M. oleifera* seeds are used in making perfumes and even in luxury mechanical devices. *Moringa* leaf powder yields are 6 t/ha/year on average, sometimes reaching 15 t under the best conditions, thus generating an average annual income of nearly 750000 CFA francs (around 1500 USD)/ha for smallholders (De Saint Sauveur & Broin, 2006; Rajangam, Azahakia, et al., 2001).

Moringa is the only genus of the Moringacae family in the Brassicales order and includes around 12 known species. *M. oleifera*, which originated in the Agra and Oudh regions of northeastern India, is the most important and widespread of these species. This subtropical species is drought resistant and adapts to many different ecological and agricultural systems. It thrives at mean temperatures between 18.7°C and 28.5°C. The plants tolerate annual rainfall levels ranging from 480 mm to 4000 mm and they grow well in near alkaline soils, tolerating soils within a broad pH range (4.5–8) (James & Duke, 1983). *Moringa* plants respond well to chemical and organic fertilization and to colonization by arbuscular mycorrhizal fungi (Pamo, Boukila, et al., 2005). Mycorrhizal colonization enables host plants to react quickly to pest infestations (Singh, et al., 2000). However, the diversity and activity of these mycorrhizal fungi are known to be affected by pH and nutrient status of the soil (Huang, et al., 1983) as well as by organic matter content (Jurgensen, Harvey, et al., 1997). These soil features have been shown to be affected by soil disturbances due to tillage and consequently the previous cropping history and vegetation cover (Lawson, et al., 1990; Onguéné, 2000).

In Cameroon, *Moringa* plant regeneration is unfortunately hampered to various extents in rainforest soils under different crop management strategies. Hence, this first study of *M. oleifera* cropping assessed the impact of previous vegetation cover on mycorrhization and development of *Moringa* plants in rainforest soils.

2. Materials and Methods

This study was carried out in the vicinity of Awae and Minkoameyos, localities from Mefou-Afamba and Mfoundi departments in the Central (rainforest) region of Cameroon. This Central region is situated between 3° 31' and 6° 54' latitude N and between 10° 46' and 12° 52' longitude E (Figure 2).The two localities were chosen as a debut of a planned study of the potential expansion area of the species' cultivation in the Central Region, with more localities to be taken into account in the subsequent phase of the project. The choice of these localities was based on the similarity of the cropping systems practiced there. Furthermore, they are close and among the main suppliers of food markets in the capital city Yaoundé.

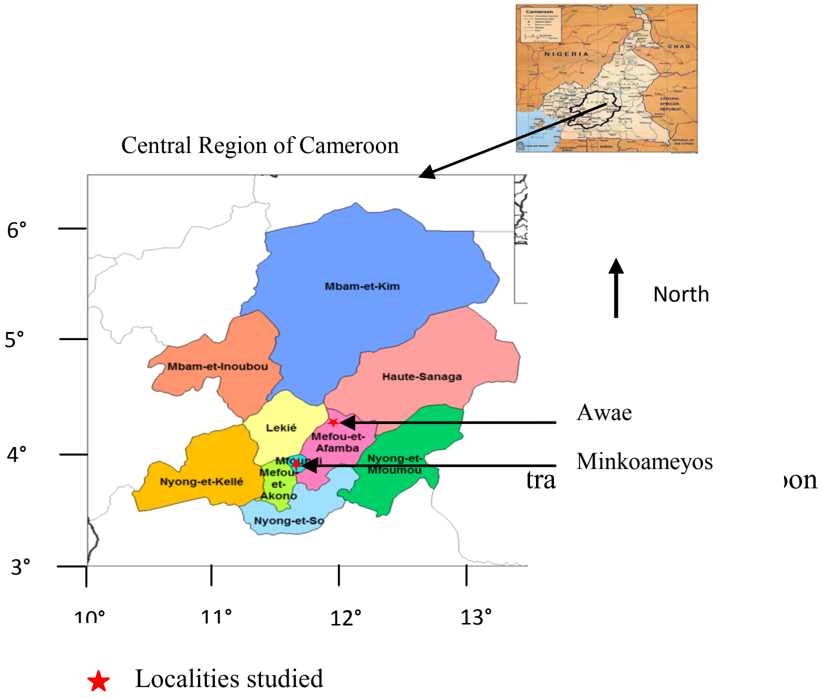


Figure 2. Localities studied in the Central Region of Cameroon

The Central region is located in southern Cameroon on a large plateau with a mean elevation of 650 m above sea level (Westphal *et al*., 1981). It is part of a closed forest agro-ecological area under a wet equatorial climate with four seasons: two rainy seasons from September to November and March to June, alternating with two dry seasons from December to February and June to August. The mean annual temperature ranges from 23°C to 25°C. The closed forest zone has 1800 to 2400 mm/year of rainfall, with 1776 h/year of sunshine on average (MINADER, 2000). Its soils are largely highly desaturated red ferralitic soils with low organic matter and exchangeable base contents. These soils have a low (10-12) cation exchange capacity (CEC) and become depleted after

2–3 years of cropping (Yerima & Van Ranst, 2005). At each study site, three previous vegetation covers were identified, i.e. forest, *Chromoleana odorata* fallow at least 3 years old, and crop field. Soil disturbance levels were considered to be (in increasing order): forest, fallow and crop field. Soil samples were collected from the top 20 cm soil layer. *M. oleifera* plants generated from germinated seeds were sown in 12 cm wide by 18 cm high polystyrene bags holding 2.5 kg of crude dry soil taken from the two sampling sites. Polybags filled with soil were maintained on an 80 cm high support for 3 months (November–February). In the two localities, soils used are red ferralitic, characterized by a clayey sandy texture with a pH between 4.0 and 5.5.The organic matter content was 1.4 - 2.6% for Awae and 2.0 – 3.5% for Minkoameyos. All plants were grown in the greenhouse located in Nkolbisson away from the sampling sites. The greenhouse was a hangar three meters high 1/3 covered with transparent sheet metal and 2/3 with aluminium sheet metal sheet, surrounded by a wall one meter high, filtering 50% of the direct solar radiation. No supplementary lighting was provided during the test. Daily watering to maintain sufficient soil moisture in the polybags was increased gradually. The experimental design was a randomized complete block design with three treatments, 4 plants per treatment and 4 replicates. The parameters monitored were the root mycorrhizal colonization rate, plant height and the above-ground biomass dry weight. After sowing, the height of each plant was measured every 2 weeks. The root mycorrhizal colonization rate and the above-ground biomass dry weight after three months i.e. the end of the experiment because these observations were destructive to the plants. The above-ground biomass dry weight and the mycorrhizal colonization rate (MCR) were determined, the biomass after 72 hr oven drying at 70°C and the MCR according to the most probable number (MPN) method (Anderson & Ingram, 1993).

The SPSS 10.1 software package was used for Levene's test of the homogeneity of variance and analysis of variance (ANOVA) for MCR, plant height and biomass as a function of the previous vegetation cover. Means of the above parameters were separated using the Waller-Duncan multiple comparison procedure. Partial correlation coefficients between previous vegetation covers and the study location were calculated.

3. Results

Very low early *M. oleifera* plant mortality was observed only in the forest soil from Awae. Around 12% mortality was recorded in all plants less than 6 weeks old planted in soil from this location, with no significant differences noted between the three types of previous vegetation cover. *M. oleifera* plant height, biomass and MCR were not correlated with the previous vegetation cover or with the number of living plants at either Awae or Minkoameyos. The recorded data were found to be homogeneous and thus did not require transformation for the remainder of the study (Table 1).

Table 1. Analysis of variance for mycorrhizal colonization rate, plant height and biomass per locality

Source of variation	Location	Fisher's values		
		MCR	Height	Biomass
Inter-group	Minkoameyos	1.5^{ns}	8.8**	6.3*
	Awae	5.1*	6.3*	4.9*

Note: ^{ns} : non-significant; * : significant at the 5% level; ** : significant at the 1% level

3.1 Mycorrhizal Colonization Rate

A microscopic analysis of roots revealed the same mycorrhizal structures (internal and external hyphae, vesicles and auxiliary cells) whatever the previous vegetation cover (forest, fallows and crops). The presence of sphere-shaped auxiliary cells with a flat surface amongst the observed mycorrhizal structures indicates that *M. oleifera* forms mycorrhizae with fungi of the *Scutellospora* genus as these are the only fungi known to form this type of auxiliary cell. On average, 72% of the observed organs were the external hyphae.

ANOVA for MCR revealed differences between the two study sites depending on the previous vegetation cover (Table 2). No differences in MCR were found when comparing the previous vegetation covers at Minkoameyos, unlike those in Awae where a significant difference was found (Table 1). The mean MCR was 34 % in the Awae samples. The highest MCR value in the Awae samples (40.5 %) was recorded in the cropped field soil, and the lowest (20.3 %) was obtained in the forest soil. The MCR values obtained at Minkoameyos were all higher than those obtained at Awae, regardless of the previous vegetation cover (Table 2).

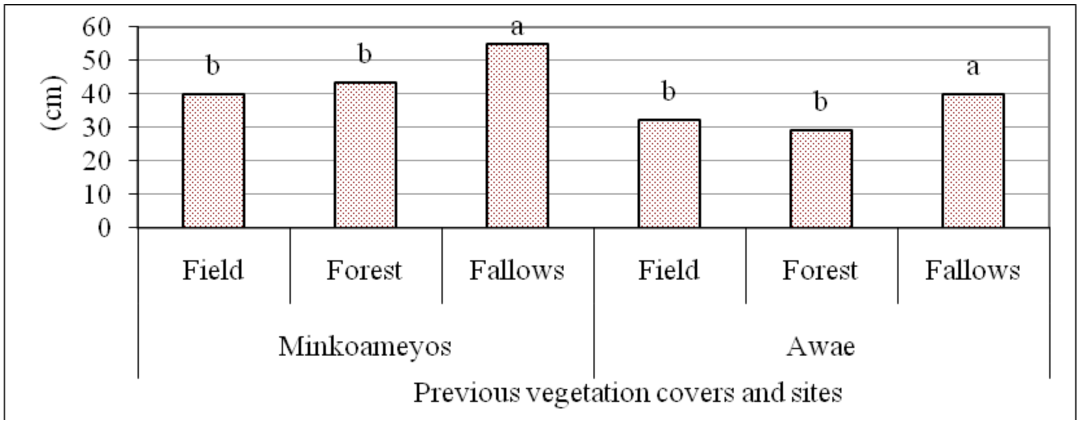
Table 2. Root mycorrhizal colonization rate values at
the two study sites according to the previous vegetation covers

Previous	Minkoameyos		Awae	
Vegetation covers	Average	Standard error	Average	Standard error
Field	51,8a	3,1	43,5a	5,7
Forest	63,3a	4,3	20,2b	4,8
Fallows	60,0a	6,7	38,3a	5,7

Note: Means with the same letter for the same site are not significantly different at 5 % level of significance.

3.2 Plant Height

Differences in plant heights were noted across the previous vegetation covers at Minkoameyos and Awae (Table 1). The highest plant heights (55.1 cm and 39.9 cm) were recorded in the fallowed soil at Minkoameyos and Awae, respectively. However, in absolute terms, the shortest plants (40.0 cm) were found in the cropped field soil at Minkoameyos but in the forest soil at Awae (30.8 cm) (Figure 3).



Note: Means with the same letter for the same site
are not significantly different at 5 % level of significance

Figure 3. Plant height after 12 weeks
at the two studied localities according to previous vegetation cover

Plant growth was ascendant during the whole duration of the trial. The superiority of fallow soils over forest and crop field soils is most observable. However, a slight overlap can be noted between the forest and field soils at Awae until week 6, after which plants in forest soils showed better growth, in absolute values, than crop field soils (Figure 4).

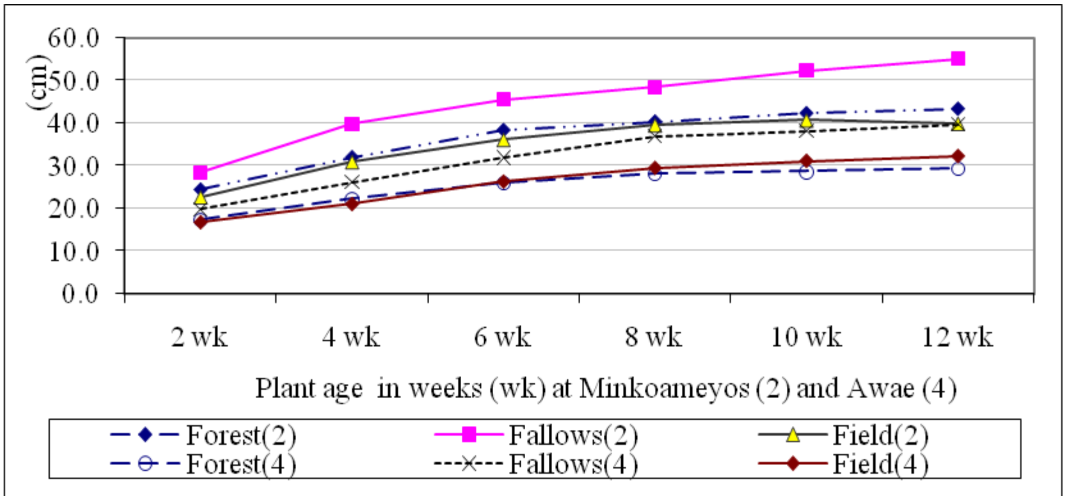
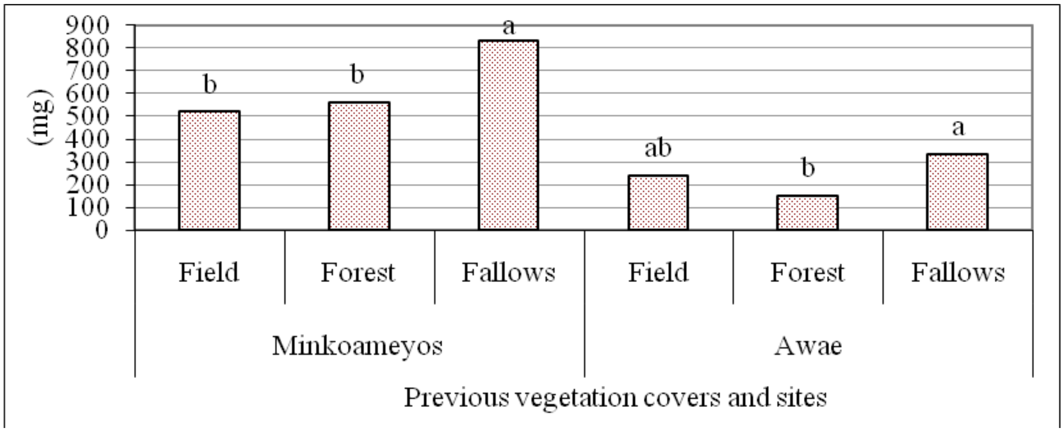


Figure 4. Plant growth in soils with different previous vegetation covers at the two studied localities according to plant age

3.3 Biomass

Differences were noted across previous vegetation covers at Awae and Minkoameyos in terms of biomass. Mean biomass production at Minkoameyos and Awae was 638.8 mg and 240.8 mg, respectively. The highest biomass production at the two study sites was recorded in plant grown in fallowed soil at both Minkoameyos (834.7 mg) and Awae (332.7 mg). The lowest biomass production for each site was recorded in the field soil at Minkoameyos (520.7 mg.) and in the forest soil at Awae (150.3 mg), in absolute terms. Biomass production was found to be better in soils at Minkoameyos, regardless of the previous vegetation cover. Based on the mean values, presented in Figure 5, biomass only from fallowed soils differed from that produced in soils with the other previous vegetation covers.



Note: Means with the same letter for the same site are not significantly different at 5 % level of significance.

Figure 5. Variations in above-ground biomass at the two studied localities according to previous vegetation cover

At Minkoameyos, there were no correlations between the MCR and biomass or between the MCR and plant height. However, positive correlations between the MCR and biomass (r = 0.653; p = 0.021*) and between the MCR and plant height (r=0.707; p = 0.011*) were obtained for the Awae samples.

4. Discussion

The early mortality observed only in the forest soil at Awae was probably due to the low suitability of its soils to *M. oleifera* growth or mycorrhizal development. Plants from Awae recorded low values for all the parameters measured. Furthermore, soils samples were not treated before planting and might have hosted pathogenic strains of fungus or bacteria that interfered with plant health and growth. It has been reported that mycorrhizal colonization enables host plants to respond quickly to pest infestations (Singh, Adholeya, et al., 2000), which is in line with the findings of Janos (Janos, 1980), who showed that the extent of available mycorrhizal fungi is a key factor in determining forest composition during the early forest growth phase. It is also known that *M. oleifera* is attacked by certain fungi such as *Cercospora* sp.*,* *Puccinia* sp.*,* *Oidium* sp. and *Sphaceloma morinda* whose interactions with mycorrhizal fungi have yet to be clearly determined.

The low MCR observed in the forest soil at Awae is additional evidence that a pathogenic fungus infestation may have been involved. Daniels & Menge (1980) have reported that the abundance of mycorrhizal fungi or their activity can be affected by other pathogenic fungi or bacteria. The low MCR generally observed in soils at Awae could equally have been due to the physicochemical properties of the soils, such as the pH and soil texture. The chemical properties of the soil have been noted to exert a much greater effect on the abundance and activity of mycorrhizal fungus than do soil disturbances due to tillage (Hayman, 1982; Mosse, 1972). The MCR recorded at Minkoameyos, but not that recorded at Awae, was similar to that obtained by Onguéné (2000), who found that mycorrhizal colonization rates in rainforests in southern Cameroon were much higher than in fallows or cropfields. A better mycorrhizal colonization rate was expected in the fallowed soil because of the suitable environmental conditions created by *C. odorata*, which generates abundant organic matter that is recycled back into the soil. This is in agreement with previous findings (Splittoesser, 1984; Keeton *et al*., 1993) that organic manure decomposition boosted the level of soil humus, consisting mainly of cellulose, hemicellulose and lignin, which represent an efficient source of energy for soil microorganisms. In the Congo, desaturated and highly acidic ferralitic soils under *C. odorata* fallow cover were found to have a higher pH, associated mainly with calcium enrichment, than that of primary or secondary forest soils (Forester & Schwartz, 1991), suggesting that soil health and chemical properties would have influenced mycorrhizal colonization at the two sites studied here.

The plant height growth curves showed that growth was rapid at the beginning of the study, with a slowdown near the end. This pattern of plant height growth may be explained first by the depletion of nutrient reserves of the seeds and the soil in the polybags depending on the previous vegetation cover, suggesting that the plants no longer had enough mineral elements for its normal growth. Crop field soils appeared to be more impoverished than forest and fallow soils. Furthermore, in soils from certain previous vegetation covers, plants may have had a low root density reducing the uptake of mineral elements. Therefore *M. oleifera* plant growth could be improved via fertilizer applications (Pamo et al, 2005) with organic fertilizers which increase the root density (Palm et al., 2001), leading to improved nutrient uptake. Fallowing contributes to restoring soil fertility. Better *M. oleifera* plant growth was observed in *C. Odorota* fallow soils, attesting to the relatively high fertility of fallow soils. Similarly, Palm *et al*. (2001) and Foidl, Makkar, et al., (2001) also observed that cow dung enhanced *M. oleifera* plant growth in Nicaragua. It has been demonstrated that *C. odorata* recycles a considerable quantity of organic matter back into the soil, thus improving the soil structure and hampering mineral leaching by limiting downward water movement in the soil (Chevalier, 1952; Van der Meulen, 1977). Organic matter, organic carbon, nitrogen and phosphorus are more available in soils fallowed under *C. odorata*. Fallows with this species thus enhance agricultural sustainability, with a reduction in fallowing time and in organic fertilization (Jibril & Yahaya, 2010).

At study sites, the plant height and biomass production findings could be explained by the previous vegetation cover and the soil physicochemical properties. What can be considered better growth and biomass production recorded in fallowed soils at both study sites would be the result of the soil fertilizing effects of *C. odorata*, which corresponds to the results of Kanmegne, Duguma, et al. (1999), who found that this species significantly increased the soil nutrient availability. These results are in line with the findings of Agoumé & Birang (2009) that the fertility of *C. odorata* fallow soils was high. Other authors (Agbim, 1987; Herren-Gemmill, 1991; Assa, 1987) also noted that *C. odorata* substantially enhanced the mineral and organic fertility of relatively infertile soils**.** However, mycorrhiza also markedly improves plant growth by promoting and increasing nutrient uptake via the roots. At equivalent fertility, soils that provide the best mycorrhization conditions have the best results for mycorrhizal plant crops (Habte & Manjunath, 1987). It can be assumed that soils at Minkoameyos provide better micorrhization conditions than those at Awae. However, having said this, *M. oleifera* introduction to Awae remains possible because soil chemical and heath conditions can be controlled using appropriate agricultural practices.

The lack of a correlation at Minkoameyos between the MCR and biomass or between the MCR and plant height could be due to the fact that other factors either that mycorrhizal colonization which have played a moderating effect of the plant response. For example, higher availability of phosphorus which contributes to the development of the root system on which plant growth depends could found at Minkoameyos and relatively insufficient at Awae where mycorrhizal colonization alone would have led to improved growth and biomass. Another contributing element can be the available water holding capacity that could have been relatively satisfactory in the soils at Minkoameyos and insufficient in the soils at Awae where it has been improved as a result of micorrhization.

This study highlighted MRC in forest, fallows with *C. odorata* and crop-filled soils, with the best growth in *C. odorata* fallow soils. *C. odorata* fallow soils appear to be suitable for *M. oleifera*'s high dry matter production and growth followed by forest soils. The chemical properties and microbiology of the soil had the stronger effect on the study parameters, particularly at Awae. Despite the relatively low MCR, soils fallowed under *C. odorata* could be recommended for growing *M. oleifera* because they provide the best conditions for plant development. Forest soils are better than crop field soils in absolute terms for MRC, dry matter production and plant growth. In practice, *C. odorata* fallow soils are allowed to rest for 3 to 5 years before a subsequent cropping cycle with the previous food crops that do not include *M. oleifera*. Furthermore, in the study localities as well as in the whole rainforest areas, *C. odorota* fallow soils occupy relatively smaller area compared to vast forest soils for the implementation of *M. oleifera*'s plantations. Crop field soils are already dedicated to desired crops and could not be easily converted to *M. Oleifera*'s plantations. Only intercropping of *M. oleifera* with other crops would be possible in fallows. Logically, the establishment of *M. oleifera* plantations or intercropping system in which *M. oleifera* would be the dominant crop will only be possible in the vast areas of forest soils of the Central region of Cameroon. In addition to more specific characterisation of the soils in Awae and Minkoameyos, future research would seek to isolate and identify local strains of fungi and bacteria that may be pathogenic to tree crops and could interfere with the growth of *M. oleifera* or/and mycorrhizal development.

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