

Effects of different cowpea (*Vigna unguiculata*) Varieties on Soil Microbial Population and Biomass Carbon

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Abstract

The study determined the effects of different cowpea varieties on soil microbial population and biomass carbon. Rhizosphere soils of six different cowpea varieties were selected based on their nodulating ability. This study selected two of the most nodulating varieties (Ife Brown and Tou113), two medium nodulating varieties (IT08K-150-27 and IT07K-230-2-9) and two least nodulating varieties (Sanzi and IRS-09-1106-4) for analysis. The laboratory was used to assess the microbial population and soil biomass carbon content. Findings indicate that the soil surrounding the roots (rhizosphere soil) of IT08K-150-27 which was of the medium nodulating variety gave the highest microbial population and biomass carbon. Thus, IT08K-150-27 outperformed other varieties in boosting soil microbial activities, consequently improving soil health. Owing to the results obtained in the study, it can be concluded that nodulation ability in cowpea might not proportionately affect soil microbial activity in the rhizosphere soils.

Keywords: Cowpea, Rhizosphere, Soil, Biomass, Microbial.

INTRODUCTION

The problem of soil sustainability has become one of the greatest challenges facing the agricultural sector today, especially in developing countries (Pawlak & Kołodziejczak, 2020), and this has brought about the need to incorporate legumes in cropping system so as to achieve a balanced food and national security (Ahmad *et al.*, 2022). In this region, cowpea

(*Vigna unguiculata* (L) walp) holds significant importance as a dietary staple (Kebede & Bekeko, 2020).

Legumes, in symbiotic association with rhizobial microorganisms have the in-built capacity to convert atmospheric nitrogen (N) in their root nodules, thus reducing the reliance on chemical form of N which is not only expensive, but also less environmentally friendly (Shome *et al.*, 2022). Legumes differ in their ability to form root-nodules with plants and fix N₂ (Mathesius, 2022). Tesfaye & Nebiyu, (2021) reported that varietal difference in cowpea influences nodulation and N₂ fixation. Part of the N₂ fixed by legumes (e.g. cowpea) can be released into the soil and be beneficial to other crops in a mix or planted following harvest of the legumes (Kebede, 2021, Carter *et al.*, 2021). Cowpea like many other plants, through their roots, do release organic compounds (exudates) which include amino acids, sugars, proteins, vitamins, nucleotides, fungal stimulators, into the soils around the roots - rhizodeposition (Upadhyay *et al.*, 2022). These chemical compounds serve as a source of nutrient to microbes around the roots (rhizosphere microbes) and result in the plant roots exerting rhizosphere effects on the microbes around it (Santoyo *et al.*, 2021). This effect can also vary with crop types or difference in varieties among same plant species (Beillouin *et al.*, 2021). Soil microbes perform various functions, including the decomposition of organic materials and the recycling of nutrients within the soil. (Raza *et al.*, 2023). They flourish and increase rapidly in the presence of nutrient source (Raza *et al.*, 2023). Hence, the rhizosphere microbes rapidly build their cell biomass and increase in population due to nutrient availability (Athuman, 2023).

Elements like nitrogen, phosphorus and sulfur that provide nourishment taken up by the microorganisms constitute part of labile soil nutrient pools that are available for plant uptake after the death of the microbes (Khoshru *et al.*, 2023). In addition, microbial biomass carbon (C) is prevented from contributing to the increase in atmospheric CO₂ during the life span of the microbes and hence, important for soil C sequestration and global C cycling (Liu *et al.*, 2021).

Thus, the importance of legumes in N₂ fixation in association with rhizobia has greatly increased the interest in the study of different legume varieties owing to their influence on soil property and nutrient management (Goyal *et al.*, 2021). Therefore, the aim of this study was to determine the effects of different varieties of cowpea on soil microbial population and microbial biomass C.

MATERIALS AND METHODS

Study Area

The research was conducted at the teaching and research farm of Obafemi Awolowo University, located in Ile-Ife, Osun State, within the Southwestern region of Nigeria. The site is within the latitudes 7°32' N and 7°33' N and longitudes 4°32' E and 4°40' E, in the rain forest zone of Nigeria. The altitude is about 200 metres above mean sea level. The annual rainfall in the area covers approximately eight months with mean of 1400 mm, bimodal in distribution pattern and peaks in June and September. The area's soil originated from coarse-grained and gneiss and was categorized as Iwo series, identified as Ultisol (Ashaolu *et al.*, 2019).

Collection of Soil Samples

Soil samples of six different cowpea varieties (Ife Brown, Sanzi, Tvu113, IT08K-150-27, IT07K-230-2-9 and IRS-09-1106-4) were obtained from OAU teaching and research farm. The selection of soil samples was determined by the nodulation capacity of each cowpea variety (Goyal *et al.*, 2021). A portion of the soil samples underwent crushing, air drying and sieving with a

2mm sieve to eliminate roots and stones. This prepared portion was then taken for laboratory analyses, while the remaining portion was utilized to assess microbial population and biomass carbon.

Sample collection and preparation

The experimental field was planted with fifty-six (56) different cowpea varieties of which 46 varieties were acquired from the Department of Crop Production and Protection at Obafemi Awolowo University, Ile-Ife, Osun State, and the remaining ten (10) were sourced from the International Institute for Tropical Agriculture, IITA, Ibadan, Nigeria. Each of the cowpea varieties were planted and replicated three times. The experiment utilized a Randomized Incomplete block design, specifically an 8 x 7 alpha lattices configuration. Replication was done using the serpentine layout method. Each experimental plot consisted of eight rows and seven columns. Each variety was planted with two seeds per hole, spaced at intervals of 75 x 25cm. Each experimental plot constituted two lines of 2m length for each variety. Each replication had a distance of 2 m length and an alley of 1 m.

At about 50% flowering stage which marked the peak of nodulation, for each variety, three to four plants were harvested by excavating a soil cube approximately 25 x 25 cm in size, extending as deep as the roots or the space allowed. The selected legume plant was carefully lifted out with the spade onto a plastic sheet. The soil around the roots was manually removed and the legume roots were gently loosened by hands. The number of nodules per plant was counted and documented. The fresh and dry weights of the nodules per stand were also established. The rhizosphere soils of the harvested plants were also collected. The rhizosphere soils of six cowpea varieties (Ife Brown, Tvu113, IT08K-150-27, IT07K-230-2-9 Sanzi and IRS-09-1106-4) were later selected for the determination of microbial population and biomass C. The selection was based on nodule numbers and weight. Thus, two each of the highest (Ife Brown and Tvu113), medium (IT08K-150-27 and IT07K-230-2-9) and the least (Sanzi and IRS-09-1106-4) nodulating varieties were selected for this study.

Distribution of Particle Size

The particle size distribution was assessed utilizing the Bouyoucocus Hydrometer method as adapted by Pawlak and Kołodziejczak (2020) using 0.2 M sodium hydroxide (NaOH) as the dispersing agent. Fifty one grams (51 g) of the air-dried soils was measured by weight and transferred into the dispersing bottles and 100 mL of 0.2 M NaOH was added. The bottles were sealed securely and shaken for 5 minutes to reach equilibrium and then transferred to a reciprocating shaker which was used to shake the suspensions for 3 hours. The suspension was then accurately transferred into a 1000mL glass cylinder and filled up to the 1000 mL level using water. A plunger was employed to stir the sample and disperse the particles evenly in the suspension. The initial hydrometer reading was recorded 40 seconds post plunger withdrawal, while the suspensions temperature was concurrently measured. After allowing the suspension to settle undisturbed for 3 hours, another hydrometer reading and temperature measurement were obtained. Reagent blank analysis was conducted alongside the samples. The distribution of primary separates (sand, silt and clay) was subsequently calculated using a specified relationship :% sand = $100 - 2 [(H_1 - B) + 0.36 (T_1 - 20 ^\circ\text{C})]$, % clay = $2 [(H_2 - B) + 0.36 (T_2 - 20 ^\circ\text{C})]$
% silt = $100 - (\% \text{ sand} + \% \text{ clay})$.

Where, H_1 = Hydrometer reading at 40 seconds, H_2 = Hydrometer reading at 3 hours, B = Hydrometer reading of the blank, T_1 = Temperature at 40 seconds, T_2 = Temperature at 3 hours
 $0.36 (T - 20 ^\circ\text{C})$ = Temperature correction that was added to hydrometer readings

Field Moisture Capacity

This was determined gravimetrically. An empty 1000 mL measuring cylinder was weighed with a vent tube inside. The cylinder was filled with soil slowly to prevent compaction until the 1000 ml mark was reached. The cylinder containing the soil was re-weighed. A layer of cotton wool was placed on the soil and 150 mL of distilled water was carefully poured in. The cylinder was then covered with polythene with ten pinholes made on it and left for 72 hours. Samples were taken from the wetting front of the soil into drying cans of known weight. The can containing the soil was weighed and then the soil was dried in an oven at 105°C until it reached a constant weight. Next, the weight of the dried soil was measured and the percentage of field moisture capacity (FMC) was calculated as follows:

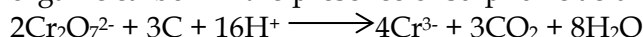
$$\% \text{ FMC} = \frac{\text{weight of wet soil (g)} - \text{weight of oven dried soil (g)}}{\text{weight of oven dried soil (g)}} \times 100$$

Determination of Soil pH

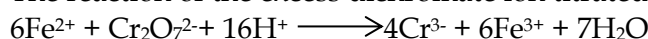
The pH was assessed using the Jaiswal 3015 electrode pH meter according to the described method by Khoshru *et al.*, (2023) that had been standardized with buffers of pH 4 and 7. Ten grams (10 g) of soil were measured into pH cups in duplicate. To one cup of soil, 10 mL of distilled water was added and to the other cup, 20 mL of CaCl₂ was added. The suspensions were left to settle for 30 minutes with intermittent stirring. The pH meter reading was then noted as the soil pH in water.

Determination of Soil organic matter

The soils organic matter content was assessed using Beillouin *et al.*, (2021) chromic acid digestion technique. 0.5 g of the air-dried soil was weighed into 500 mL Erlenmeyer flask. Ten millilitres (10 ml) of 0.5 M K₂Cr₂O₇ solution was added and the solution was swirled to mix. Twenty millilitres (20mL) of concentrated H₂SO₄ was added and the mixture was allowed to sit for 30 minutes. The suspension was then diluted with 200 mL of distilled water followed by the addition of 10 ml of 85% H₃PO₄, 0.2 g NaF and 1 ml of diphenylamine indicator. The excess Cr₂O₇²⁻ was back titrated with 0.5 M ferrous solution to a green end point. A reagent blank was carried out alongside the samples. The reaction of the dichromate ion (Cr₂O₇²⁻) with organic carbon in the presence of sulphuric acid is expressed as follows:



The reaction of the excess dichromate ion titrated with ferrous ion is:



The amount of organic carbon and organic matter in the soil was calculated using the following equations:

$$\text{Milliequivalent of readily oxidizable material per gram of soil (meq. Ox/g)} \\ = \frac{(\text{ml of Fe}^{2+} \text{ for blank} - \text{ml of Fe}^{2+} \text{ for sample}) \times \text{Normality of Fe}^{2+}}{\text{Weight of soil (g)}}$$

$$\% \text{ organic carbon} = \text{meq.Ox/g} \times 12/4000 \times 1/0.77 \times 100$$

$$\% \text{ organic matter} = \% \text{ carbon} \times 1/0.58 = \% \text{ carbon} \times 1.72$$

Where,

12/4000 = milliequivalent weight of carbon in grams

1/0.77 = factor for converting carbon actually oxidized to organic carbon

100 = factor to change from decimal fraction to percentage

1/0.58 = factor for converting organic carbon to organic matter, with the assumption that organic matter contains 58% carbon.

Determination of Total Nitrogen

This was determined using the micro Kjeldahl digestion and distillation procedure (Beillouin *et al.*, 2021). One gramme (1 g) of soil, dried naturally, was weighed and placed into an 11 cm Whatman No. 2 filter paper, wrapped and inserted into a 500 mL Kjeldahl flask. One teaspoon of digestion mixture was introduced into the flask followed by the addition of 20 mL of concentrated H₂SO₄. The flask was then gently swirled until the sample and acid were completely mixed. This was then digested at low heat in the Kjeldahl digestion apparatus for 3 hours. The flask was then removed from the apparatus and left to cool. Afterward, the solution was transferred into a 50mL volumetric flask and diluted to the mark with distilled water. Next, 20 mL of the solution was moved into the distilling flask, followed by the gradual addition of 50 mL of 40% NaOH. The blend was then steam distilled into a 4% boric acid indicator solution in a 250 mL conical flask using the Kjeltex system 1002 distilling unit. Once approximately 150 mL of the mixture had been distilled, the resulting distillate was titrated against 0.1 N HCl until the blue color vanished. The percentage of total nitrogen was determined as follows:

$$\% \text{ Total N} = \frac{[\text{sampe titre (mL)} \times \text{Normality of HCl} \times 0.014 \times \text{dilution factor} \times 100]}{\text{Sample weight (g)}}$$

Determination of Available phosphorus

The available phosphorus was determined using the Bray-1 method Kebede & Bekeko, (2020). Two grams (2 g) of soil sample, previously dried in air, were measured into a 50 mL conical flask. Then, 20 mL of P-A solution (0.025 N HCl + 0.03 N NH₄F) was added and the mixture was shaken for 5 minutes. The resulting suspension was promptly filtered through a 9 cm Whatman No. 2 filter paper into a laboratory test bottle. After filtration, 3 mL of the filtered aliquot was transferred into a colorimeter tube, followed by the addition of 3mL of P-B solution (0.87 N HCl, 0.38% ammonium molybdate, 0.5% H₃BO₃). Subsequently, 5 drops of P-C solution (8 g of P-C powder dissolved in 50mL warm distilled water) were added to the tube, mixed, and allowed to stand for 15 minutes to develop the blue color of the complex. The absorbance of phosphorus (P) was measured on the colorimeter, previously calibrated with standard P solutions, at a wavelength of 660 nm. A standard curve was generated by plotting absorbance against concentrations of P in the standard solutions. Finally, the concentration of P in the sample was determined using this standard curve.

Determination of Exchangeable cations

The concentrations of Na and K in the soil samples were determined via flame photometer FP 640 model, while Ca and Mg were determined using Atomic Absorption Spectrophotometer (Khoshru *et al.*, 2023). Ten grams (10 g) of soil sample, previously dried in air, were measured into a 250 mL conical flask. Then, 100mL of 1 NH₄OAc (ammonium acetate extraction solution) was added. The mixture was shaken intermittently for 30 minutes and then filtered through Whatman No. 1 filter paper into a laboratory test bottle.

Estimation of Microbial Population

Microbial population assessment was conducted for both heterotrophic bacteria and fungi in the soil. To determine total heterotrophic bacterial (THB), one gram of each soil sample was measured and placed into a test tube containing 10 milliliters of sterile distilled water. Thereafter, 0.1ml of the suspension was transferred into 9.9mL sterile distilled water in another test tube to obtain a hundred-fold (10⁻²) serial dilution. A measure of 0.1mL was taken from the dilution into another 9.9 ml sterile distilled water, and this process continued till a millionth (10⁻⁶) fold serial dilution was obtained. One milliliter (1.0mL) of each dilution was then plated in sterile Nutrient Agar (NA) medium in sterile petri dishes using the Pour Plate method. Each plate was swirled in the clockwise, anticlockwise, left and right directions for

homogeneity and then allowed to cool and set. The culture plates were placed in an incubator set to a temperature of 35°C for a period of 24 hours. Following this incubation period, plates containing colonies numbering between 30 and 300 were chosen for enumeration. The mean viable counts were then adjusted by the dilution factor and presented as the quantity of colony-forming units (CFUs) per gram of the soil sample. The Total Heterotrophic Fungi was determined following the same procedure as for bacteria, but Potato Dextrose Agar (PDA) was used instead of NA and the growth of bacteria was inhibited by streptomycin. Also, the plates were incubated at 28 °C for 3 to 5 days until constant colony numbers were obtained (Khoshru *et al.*, 2023).

Estimation of Microbial Biomass Carbon

This was measured using the chloroform fumigation-extraction method (Beillouin *et al.*, 2021). The chloroform was washed with concentrated H₂SO₄ and distilled water to remove the ethanol. Each soil sample, weighing five grams, was distributed into two 50mL centrifuge tubes labeled A and B. To each tube, 20mL of 0.5 M K₂SO₄ solution was added, with an additional 0.5mL of ethanol-free chloroform included in tube B. After capping, the tubes were simultaneously shaken for one hour. Subsequently, the suspensions were allowed to settle for 10 minutes before filtering the supernatants through Whatman No. 42 filter paper. For the chloroform-treated sub-samples, only the top 15 ml of the supernatant was filtered to minimize chloroform content. Following filtration, the filtrates were aerated with air for 30 minutes to eliminate residual chloroform, with blank samples treated similarly. Moisture content analysis was conducted concurrently with the experiment. Carbon content determination was carried out using the chromic acid digestion method.

RESULTS AND DISCUSSION

Table 1 displays the physical and chemical properties of the soil utilized in the experiment. According to the USDA soil textural triangle, the soil was classified as sandy loam with a field moisture capacity of 15.68%. It exhibited a very acidic pH (Akinde *et al.*, 2020) of 3.56 in 0.01M CaCl₂ and contained a moderate organic carbon content of 11.9g/kg (Xing *et al.*, 2022). Additionally, the soil demonstrated very low levels of exchangeable Ca²⁺, Mg²⁺, and K⁺ at 0.06, 0.11, and 0.06 cmol/kg respectively, along with an exchangeable Na⁺ level of 0.01cmol/kg. These low exchangeable cation levels and the acidic nature of the soil indicate nutrient depletion.

Table 1: Physical and chemical properties of the soil used for the experiment

Properties	Values
Particle Size Distribution (g/kg)	
Sand	781.8
Silt	52.8
Clay	165.4
Texture	Sandy loam
Field Moisture Capacity (%)	15.68
pH (1 : 2 0.01 M CaCl ₂)	3.56
Organic Carbon (g/kg)	11.9
Total N (g/kg)	0.12
Available P (mg/kg)	10.91
Exchangeable Cations (cmol ⁺ /kg)	
Potassium (K)	0.06
Calcium (Ca)	0.11
Magnesium (Mg)	0.06
Sodium (Na)	0.01

Figure 1 shows the mean microbial population in the rhizosphere soils of the six different cowpea varieties. The average microbial populations varied significantly ($p < 0.05$) among the different varieties, with bacterial counts generally surpassing those of fungi. The result showed that the total heterotrophic bacteria and fungi in the rhizosphere soils of IT08K-150-27 and IT07K-230-2-9, respectively, were significantly than those of other varieties, having 2.79×10^7 and 4.6×10^6 CFU/g of soil for bacteria and fungi, respectively. This indicated that the two cowpea varieties exerted more effects on the rhizosphere microorganisms than other varieties. Type or variety difference among crops has been reported to affect microbial activities in the rhizosphere soils (Zhang *et al.*, 2023).

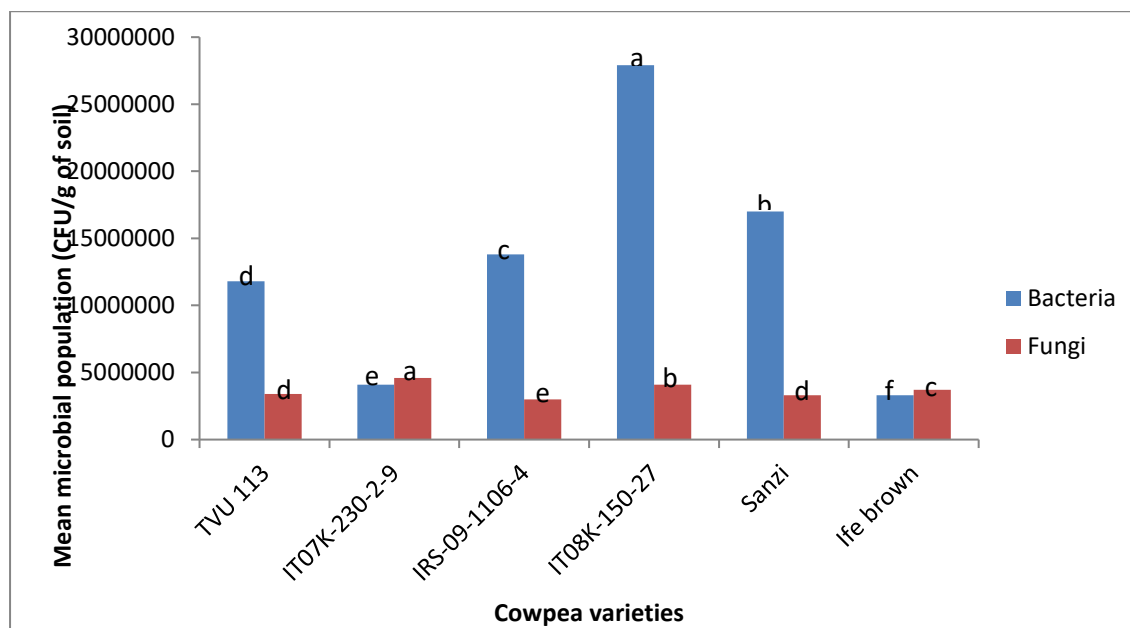


Figure1: Mean microbial population (CFU/g of soil) in rhizosphere soils of the different cowpea varieties.

Figure 2 shows the mean microbial biomass carbon in the rhizosphere soils of six different cowpea varieties. The rhizosphere of soils of IT08K-150-27, TVU 113 and Ife brown contained significantly larger biomass C than those of other varieties. This performance of IT08K-150-27 was thus similar on both biomass C and microbial population. The two cowpea varieties thus, exercise more influence on the activities of rhizosphere microorganisms.

Meanwhile, IT07K-230-2-9, Sanzi and IRS-09-1106-4 contained the lowest biomass C in their rhizosphere soils and were not significantly different from one another.

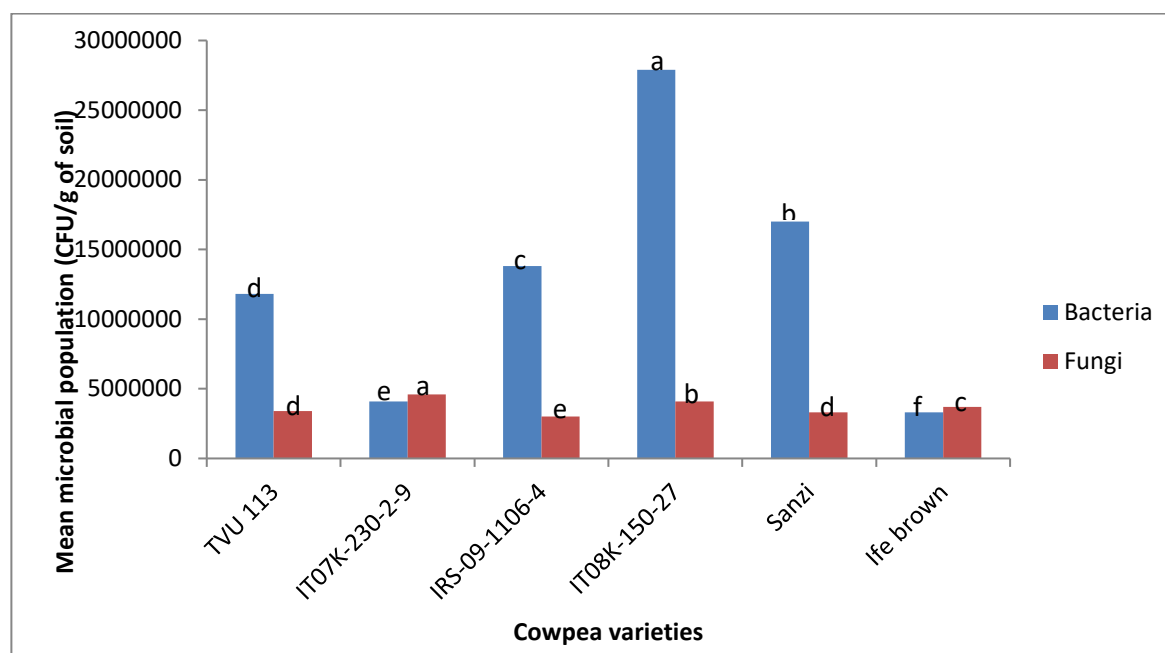


Figure 2 : Mean microbial biomass carbon C in rhizosphere soils of the different cowpea varieties.

CONCLUSION

The microbial population and biomass C were not proportionately increased with the nodulation ability of the cowpea. The cowpea variety, IT08K-150-27 which was of medium nodulating capability was superior to other cowpea varieties at enhancing the measured soil microbial activities, and consequently, soil health. It could, thus be utilized for revitalizing biologically degraded soil.

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