The use of soil biostructures created by soil fauna ecosystem engineers fed with different organic materials as inoculum source of arbuscular mycorrhiza fungi on cocoa seedling

Laode Muhammad Harjoni Kilowasid*, Muhammad Fahy Sanjaya, Laode Sabaruddin, Rachmawati Hasid, Darwis Sulaeman, Andi Nurmas

Department of Agrotechnology, Faculty of Agriculture, Halu Oleo University, Indonesia

ARTICLE INFO

| Keywords Info | ABSTRACT |
|---------------|-----------|
| Ants | Soil fauna as ecosystem engineers has the capacity to create soil biostructures, with the capacity to save arbuscular mycorrhizal fungi (AMF) spores. This study, therefore, aimed at investigating the AMF spore density in the biostructures created by cooperation between earthworms and ants with a different organic matter composition, and to analyze the biostructures' potential as a source of AMF inoculum on cocoa seedlings. In the first experiment, a combination of earthworms (0, 10, or 20 pieces) and ants (0, 10, or 20 pieces) composition, as well as a mixture of *Giricidia sepium* leaves (GSL), cocoa shell bean (CSB), and sago dregs (SD) (w/w) was tested. Meanwhile, in the second experiment, the effect of biostructures on cocoa seedlings grown in unsterile soil was examined. According to the results, the highest (46.67±13.65) AMF spore density was obtained using 20 earthworms+10 ants with 50%GSL+50%CSB+0%SD treatment, the lowest (12.67±3.78) spore count was obtained using 20 earthworms+10 ants with 25%GSL+25%CSB+50%SD. The total AMF spores were positively correlated (r² = 0.74) with the total P, but negatively correlated (r² = -0.53) with the C/N ratio. Therefore, biostructure application increased AMF spores number in the rhizosphere and the percent infection. Furthermore, biostructures resulting from the collaborative activity between different soil fauna ecosystem engineers were able to facilitate the germination of AMF spores and infect plant roots growing in non-sterile soil. |
| Earthworm | Number |
| Infection | Spore |

Article history
Submitted: 2021-05-26
Accepted: 2021-12-03
Available online: 2021-12-29
Published regularly: December 2021

* Corresponding Author
Email address: lohardjoni2@yahoo.co.id

How to Cite: Kilowasid, L.M.H., Sanjaya, M.F., Sabaruddin, L., Hasid, R., Sulaeman, D., Nurmas, A. (2021). The use of soil biostructures created by soil fauna ecosystem engineers fed with different organic materials as inoculum source of arbuscular mycorrhiza fungi on cocoa seedling. Sains Tanah Journal of Soil Science and Agroclimatology, 18(2): 166-176.
https://dx.doi.org/10.20961/stjssa.v18i2.51500

1. INTRODUCTION

Soil fauna as an ecosystem engineer has the capacity to create a new aggregate of soil containing numerous microbes useful for agriculture, forestry, and the environment (Jouquet et al., 2012). A study by Taylor et al. (2019) categorized earthworms and ants as soil ecosystem engineers. New aggregates, also known as biostructures, are created from these organisms’ activities while mixing organic matter with soil mineral particles (Zanella, Ponge, Topoliantz, et al., 2018). The form of the biostructure created depends largely on the type of soil fauna concerned (Forey et al., 2018). Biostructures formed from the activity of earthworms are known as cast, while counterparts generated by the activity of ants are known as nests or soil mounds (Bottinelli et al., 2015; Cunha et al., 2016). Both types have the ability to provide energy-rich substrates containing signaling molecules, to trigger soil microbial activity and growth of arbuscular mycorrhiza fungi (Kilowasid et al., 2015; Lavelle et al., 2016; Schultz et al., 2015; Shukla et al., 2016; Wills & Landis, 2018). The symbiotic mutualistic association between AMF and crop roots contributes significantly to expansion in the range of rooting, in order to access soil nutrients and water, and consequently, help plants meet growth needs (Powell & Bagyaraj, 2017).

The use of AMF as biological technology, in soil quality and fertility improvement, has the potential to minimize commercial N and P fertilizer use in the production of food crops, plantation seedlings, and horticulture (Cobb et al., 2018). Indonesia is known as one of the world's cocoa bean producers, where cocoa beans are produced from small farmer plantations. Generally, the farmer's cocoa trees are old, and production continues to decline (Directorate General of Estates, 2019). Thus, there is a need to replace aging plants, with seedlings that have the ability to adapt to poor soil

STJSSA, p-ISSN 1412-3606 e-ISSN 2356-1424 http://dx.doi.org/10.20961/stjssa.v18i2.51500
fertility and low groundwater availability. According to Bahrun et al. (2018), most small farmers grow cocoa seedlings in polybags filled with unsterilized. Adaptation to poor soil fertility and water deficit conditions of cocoa seedlings can be further improved by the application of AMF spore inoculum (Moreira et al., 2018; Seutra Kaba et al., 2021). Azizah Chulan (1991), reported the inoculation of AMF spores (Scutellospora calospora) produced from a mixture of soil and infected root pieces significantly increased nutrient absorption and growth in cocoa seedlings grown in sterilized soil.

The supply of soluble carbon in the substrate significantly determines AMF spores’ ability to germinate and produce infective spores under conditions in the absence of plant roots (Kameoka et al., 2019; Rillig et al., 2020; Sugiru et al., 2020). In addition, AMF’s sporulation and ability to colonize the roots are largely determined by the ratio of N:P substrates (Mei et al., 2019). Earthworms and ants are able to jointly create soil biostructures with higher microbial activity, compared to the soil not influenced by the organisms’ activity (Franco et al., 2017; Kilowasid et al., 2015). The dissolved carbon, N, total P, pH, and soil microbial population contents from biostructures produced by the activities of earthworms and ants, are strongly influenced by the type of organic matter consumed by the organisms (Ehle et al., 2019; Wang et al., 2019; Zanella, Ponge, & Briones, 2018). Fresh biostructures created by earthworms (Lumbricus terrestris L.) and black ants (Camponatus compressus Fabr.) contain populations of infective mycorrhiza spores (Harinikumar & Bagyaraj, 1994; Lee et al., 1996). However, further studies on the biostructure potential of ecosystem-engineered soil fauna to develop inoculum mycorrhiza arbuscular infective production system options for effective application at the farmer level in tropical environments, are required. Thus, this study aimed to study the AMF spore density in biostructures created by cooperation between earthworms and ants on soil enhanced with a composition of different organic matter types, as well as to analyze the biostructures’ potential, as an inoculum source of AMF spore on cocoa seedlings.

2. MATERIAL AND METHODS

Two experiments were conducted in this study. The first was to study the AMF spore density in biostructure created by cooperation between earthworm and ants on soil enhanced with a composition of different organic matter types. On the other hand, the second experiment aimed to analyze the biostructures’ potential, as an inoculum source of AMF spore on cocoa seedlings.

2.1. First Experiment

2.1.1. The site and experimental design

This first experiment was performed under standing cocoa trees in smallholder plantations from October to January 2018, located at 122°31’10.0” E; 04°08’20.5” S, and 60 m above sea level, in Tanea Village, Konda District, Konawe Selatan Regency, Southeast Sulawesi, Indonesia. In this experiment, fifteen different treatments (Table 1) of earthworm and ant (individual/individual) proportions, and with a mixture of three different organic matter (OM) types (weight: weight: weight) were tested. Organic matter includes Gliricidia sepium leaves (GSL), cocoa bean shell (CBS), and sago dreg (SD) on three levels. Soil fauna had three levels: 0, 10, or 20 individuals per reactor while OM type had also three (0, 25, and 50%) levels. Each treatment had three replicates, following a simple randomized complete block design (RCBD).

2.1.2. Preparation and application of the treatments

Earthworms (Peryonex sp.) were obtained from riverbanks around the field experiment station, Faculty of Agriculture, Halu Oleo University (located in the Kampus Hijau Bumi Tridharma, JL. H.E.A. Mokodompit, Kendari 93232 Indonesia), while ants (Dorylus sp.) were collected from smallholder cocoa plantations (located in the Dusun 3, Tanea Village, Konda District, Konawe Selatan Regency, and owner of the plantation is Pak Sapari), both through hand sorting techniques. Sago dregs were collected from a sago processing area (located in the Moramo district, Konawe Selatan Regency), cocoa bean shells were obtained from Kalla Kakao Industry (located in the Ranomeeto district, Konawe Selatan Regency), and glicidica (G. sepium) leaves were pruned from the home garden located in the Kambu district, Kendari City.

Table 1. The treatment combinations of soil fauna proportions and mixtures of three different organic matter types.

| Symbol of treatments | Description |
|----------------------|-------------|
| F0B0                 | without soil fauna and organic matter |
| F1B0                 | 10 earthworms+20 ants and without organic matter |
| F1B1                 | 10 earthworms+20 ants with 25% GSL+50% CBS+25% SD |
| F1B2                 | 10 earthworms+20 ants with 50% GSL+25% CBS+25%SD |
| F1B3                 | 10 earthworms+20 ants with 25% GSL+25% CBS+50% SD |
| F1B4                 | 10 earthworms+20 ants with 50% GSL+25% CBS+50% SD |
| F1B5                 | 10 earthworms+20 ants with 0% GSL+50% CBS+50% SD |
| F1B6                 | 10 earthworms+20 ants with 50% GSL+0% CBS+50% SD |
| F2B0                 | 20 earthworms+10 ants and without organic matter |
| F2B1                 | 20 earthworms+10 ants with 25% GSL+50% CBS+25% SD |
| F2B2                 | 20 earthworms+10 ants with 50% GSL+25% CBS+25% SD |
| F2B3                 | 20 earthworms+10 ants with 25% GSL+25% CBS+50% SD |
| F2B4                 | 20 earthworms+10 ants with 50% GSL+50% CBS+0% SD |
| F2B5                 | 20 earthworms+10 ants with 50% GSL+0% CBS+50% SD |
| F2B6                 | 20 earthworms+10 ants and 0% GSL+50% CBS+50% SD |

Remarks: GSL = G. sepium leaves; CBS = cocoa bean shell; SD = sago dreg.
All the collected materials (sago dregs, cocoa bean shells, and leaves of G. sepium) were oven-dried, pulverized using a kitchen blender, and sieved in a wire mesh with a <2 mm pore opening (Sanjaya et al., 2020). One sample of each OM was analyzed for chemical attributes include organic carbon using Walkley-Black method, total-N using the Kjeldahl method, C/N ratio, total-P, and total-K prepared using the wet-digestion method (Vogt et al., 2015). Table 2 shows the chemical attributes of each collected organic material.

Furthermore, soil (0-10 cm depth) was obtained from a smallholder farmer cocoa plantation aged over 15 years old in the Konda District, South Konawe regency. Subsequently, the soil samples were wind-dried and sieved using a < 4 mm sieve pore opening. A total of 100 g of each OM composition (treatment) was then mixed with 1.5 kg of soil in a block-shaped reactor comprised of a multiplex board, measuring 25 cm x 21 cm x 21 cm (Figure 1). The substrate in each reactor was then watered with tap water until saturated and left until no water drips through the five small holes on the reactor’s bottom surface (Sanjaya et al., 2020). It took about 24 hours to get the substrate moisture condition that allow the mobility of ants on the substrate surface in the reactor.

Earthworms measuring 5-6 cm in length were released on tissues paper surface moistened with tap water and left until no more casts were released from the anus. After emptying stomach contents, earthworms with an individual number according to the treatment (Table 1) were released into each soil surface’s of the mixture of SD, CBS, and GSL referred onward as organic material. Subsequently, the number of individual ants according to the treatment (Table 1) were released and allowed to enter into the substrate, after the earthworms’ bodies were wholly entered into the substrate. Each reactor’s entire surface was then covered with a <2 mm wire mesh green plastic gauze to prevent earthworms and ants from escaping to leave the reactor and to prevent other organisms from accessing the reactor (Figure 1). This was followed by placing all reactors on plank pads 40 cm above the ground in a simple structure with a sago leaf roof built under ±10-year-old cacao stands (Figure 2). Moisture content (about 35% measured with a soil moisture meter) was maintained by spraying 50 ml of tap water on the entire surface of the soil mixture’s every two days.

2.1.3. Estimation of total AMF spore in the biostructures

After 28 days of incubation, the soil was removed from the reactor, then earthworms and ants were separated from the soil by hand-sorting technique. The soil was air-dried under room conditions, where soil samples were taken to assess the total AMF spores and each reactor’s structural chemistry. In addition, a total of 50 g of biostructure from each reactor was poured into a container containing 500 ml of tap water and stirred to obtain a homogeneous mixture. The suspension was then filtered using 3 series of sieves with sizes of 2 mm, 200 µm, and 38 µm, from top to down, respectively. Materials retained on the 200 µm and 38 µm filters were poured into the tubes containing 20%/60% concentration of sugar solution. At the end of the spin, the supernatant was poured onto the 38 µm sieve and rinsed with water until the sugar solution was drained from the sieves. The retained spores were transferred into plastic Petri dishes (with 85 mm in diameter) with an arrangement of gridline (size 1 mm x 1 mm), and counting of AMF spores was done under a dissecting microscope (INVAM, 2019). The total number of AMF spores in each biostructure was then estimated following instructions stated in the INVAM.WVU.EDU. Briefly, the number of AMF spores was counted in 20 randomly selected field views.

![Figure 1: Reactor used for the experiment](image)

2.1.4. Chemical attribute of organic matter

Table 2 shows the chemical attributes of each collected organic material.

| Organic matter type       | Organic Carbon (%) | Total-Nitrogen (%) | C/N ratio | Total-Phosphorus (%) | Total-Potassium(%) |
|---------------------------|--------------------|--------------------|-----------|-----------------------|-------------------|
| Cocoa shell bean          | 2.62               | 0.58               | 4.52      | 5.32                  | 106.42            |
| G. sepium leaf powder     | 2.52               | 1.03               | 2.45      | 5.09                  | 114.16            |
| Sago dregs                | 2.73               | 0.53               | 5.16      | 3.57                  | 8.08              |

![Figure 2. Reactors placed inside sago hut under ±10-year-old cacao stands](image)
Kilowasid et al.  

**Table 3.** Soil physicochemical character of soil growing medium where cocoa seedlings were planted.

| Parameter             | Method         | Unit    | Value  |
|-----------------------|----------------|---------|--------|
| Sand                  | Pippete        | %       | 38.19  |
| Silt                  | Pippete        | %       | 36.69  |
| Clay                  | Pippete        | %       | 25.21  |
| pH_{H_2O (1:5)}       |                |         | 6.81   |
| pH_{KCl (1:5)}        |                |         | 6.14   |
| C-org                 | Walkey & Black | %       | 2.77   |
| Total-Nitrogen        | Kjeldahl       | %       | 0.49   |
| Total-Phosphorus      | NH_4OAc (pH 7.0) | cmol/kg | 8.27   |
| Available-Phosphorus  | NH_4OAc (pH 7.0) | cmol/kg | 2.77   |
| Ca                    | NH_4OAc (pH 7.0) | cmol/kg | 2.46   |
| Mg                    | NH_4OAc (pH 7.0) | cmol/kg | 0.21   |
| K                     | NH_4OAc (pH 7.0) | cmol/kg | 16.52  |
| Na                    | NH_4OAc (pH 7.0) | cmol/kg | not detected   |
| CEC                   | NH_4OAc (pH 7.0) | cmol/kg | 82.98  |
| Base saturation       | KCl 1N         | cmol/kg | 0.13   |
| Al                    | KCl 1N         | cmol/kg | 65.18  |
| H                     | KCl 1N         | cmol/kg | 3.60   |
| Fe                    | DTPA           | Ppm     | 32.48  |
| Cu                    | DTPA           | Ppm     | 69.99  |
| Zn                    | DTPA           | Ppm     | 6.81   |
| Mn                    | DTPA           | Ppm     | 8.27   |

The total field views were determined based on the ratio of the area of the plastic petri dish to the view field area of the ocular dissecting microscope at a magnification, where the AMF spores can be distinguished from other objects. The total AMF spores in suspension were estimated utilizing the average of AMF spores per field view multiplied with the total field views in plastic Petri dishes used. Furthermore, the total AMF spores were expressed as total AMF spore per biostructure weight where the spores were extracted.

2.1.4. Chemical characterization of the soil biostructures

The remaining biostructures’ in the reactors were wind-dried for 48 hours inside a well-ventilated room, then sieved using a <4 mm sieve pore opening, and subjected to chemical analysis, including pH using a pH meter with a 1:5 (w/v) soil-water ratio, C-org using the Walkley-Black method, total-N using the Kjeldahl method, C/N ratio, total-P and total-K using a 25% HCl extractor (Vogt et al., 2015).

2.2. Second Experiment

2.2.1. The site and experimental design

The second experiment was conducted in Tanea Village, Konda District, Konawe Selatan Regency, Southeast Sulawesi, from February to May 2019. In this experiment, physiologically ripe criollo cocoa pods were obtained from local farmers’ cocoa nurseries in Konawe Selatan Regency, Southeast Sulawesi. Cocoa beans were removed from the pods, and pithy, healthy, large beans (about 2.5 cm in length) were selected. The seeds were mixed with ashed rice husk, kneaded until the pulp of the seed’s surface coating was exposed, cleaned under running water, then the cleaned seeds were laid on the carbonized rice husk to germinate, and left to grow until roots emerged.

Table 3 shows the physicochemical characteristics of the soil used as a growing medium in this study. For this experiment, a total of 500 g of soil from the cocoa plantation was mixed with goat manure in a 2:1 ratio (v/v), placed in a polybag, and soaked in a container filled with water, until the entire surface had been inundated. The bags were removed from the container after air bubbles had stopped emerging from the soil and then left overnight. A total of 100 g of each biostructure produced from the first experiment was spread on the soil surface on each polybag. The germinated three-day-old cocoa seedlings were transplanted into polybags filled with soil+goat manure and biostructure, produced from the reactor. The experiment was established in the nursery following RCBD with three replicates. The nursery is made of wood, with a roof made of woven sago leaves, and <2 mm mesh nylon net walls. The seedlings were maintained for 12 weeks.

2.2.2. Growth measurement of cocoa seedling

At 2, 4, 6, 8, 10, and 12 weeks after planting (WAP), the plants’ height was measured, and the leaves were counted. At the end of the experiment, the seedlings were separated into shoot and root parts. The shoots were oven-dried at 60°C for 48 hours, and the dry weight was measured.

2.2.3. Estimation of total AMF spore from rhizosphere and root infection

Soil samples were obtained from the rhizosphere area for total AMF spore counting, using the procedure mentioned above. Meanwhile, the roots were washed with tap water, soaked in 10% KOH, and oven-dried at 90°C for 20 minutes. The roots were then rinsed with water, immersed in \( \text{H}_2\text{O}_2 \) for 12 hours, and the procedure was repeated once again. Roots washed from the \( \text{H}_2\text{O}_2 \) were stained in 0.05% aniline blue solution, and AMF infection on stained root pieces were observed under a microscope (Dhar & Mridha, 2012).
Figure 3. Number of AMF spores in biostructure produced from different treatments.

Table 4. Bivariate Spearman’s r² correlation (p < 0.05) between the AMF spore number and the biostructure’s chemical attribute.

| Attribute | pH  | Organic C | Tot-N | C/N | Tot-P | Tot-K | AMF spore |
|-----------|-----|-----------|-------|-----|-------|-------|-----------|
| pH        | -0.41 | -0.41 | 0.19 | 0.00 | -0.11 | -0.12 |           |
| Organic C | 0.46  |         |       | 0.33 | 0.16  | 0.24  |           |
| Tot-N     |       |         | -0.84 | 0.29 | 0.46  | 0.40  |           |
| C/N       |       | -0.28 |       | -0.49 | -0.53 |       |           |
| Tot-P     |       |       |       | 0.35 | 0.74  |       |           |
| Tot-K     |       |       |       |       |       | 0.25  |           |

2.3. Statistical Analysis

The data were subject to analysis of variance, and the means of treatments were compared using Duncan’s Multiple Range Test (DMRT) also correlation test, at a significance level of p <0.05.

3. RESULTS

The results showed a significant difference in the number of AMF spores within biostructures created by collaborative activity between earthworms and ants fed with a mixture of three types of OM (Figure 3). Soil from the cocoa plantation treated with F2B4 (20 earthworms+10 ants with 50%GSL+50%CBS and no SD) treatment produced a biostructure containing the highest total AMF spores (47 spores / 50 g of biostructure) (Figure 3). The total AMF spores in biostructures produced from the F2B4 treatment differed significantly from other treatments (Figure 3). The lowest spore count was obtained in F2B3 (20 earthworms+10 ants with 25%GSL+25%CBS+50%SD) (Figure 3).

Spearman’s rs bivariate correlation showed the number of AMF spores had a significant positive correlation with total P (r² = 0.74 at p <0.05), but was insignificant with pH (r² = -0.12), total N (r² = -0.40), C-org (r² = 0.24), total K (r² = 0.25), and with negative (r² = -0.53) correlation with C/N ratio at p> 0.05 level (Table 4).

The height of cocoa seedlings treated with soil without soil fauna and organic matter (control) was higher, as compared to counterparts treated with soil from biostructure (Table 5). At 2 and 4 weeks after application, the height of cocoa seedlings inoculated with F0B0 was the tallest (9.88±2.14cm and 20.54±2.69cm, respectively) and this differed significantly (Table 5) with F1B5 (10 earthworms+20 ants with 50%GSL+0%CSB+50%SD) and F1B0 (10 earthworms+20 ants and without organic matter) treatments, but comparable with the other treatments (Table 5). At 6 weeks after biostructure application, there was a significant difference (Table 5) in height, between F0B0 (control) seedlings, applied with biostructures in treatments F1B5 (10 earthworms+20ants with 50%GSL+0%CSB+50%SD) and F2B1 (20 earthworms+10 ants with 25%GSL+50%CBS+25%SD) (Table 4). At 8 weeks after application, a significant difference in seedling height was observed between the control, as compared to F1B5 (10 earthworms+20ants with 50%GSL+0%CSB+50%SD) and F2B1 (20 earthworms+10 ants with 25%GSL+50%CBS+25%SD) treatments. This observation on F1B5 and F2B1 treated seedlings was carried over at weeks 10 and 12.

The number of leaves of cocoa seedlings at 2, 6, 8, 10, and 12 weeks after biostructure application followed the same trend as in plant height (Table 6). Cocoa seedlings treated in F0B0 biostructure, gave consistently the highest number of leaves throughout the 12 week observation period except at week 4 where there was no significant effect of the treatment on the number of leaves of cocoa seedling grown in the 15 treatment combinations (Table 6).
Table 5. Cocoa seeding height (cm) during the twelve weeks growth period, after biostructure application.

| Biostructure sources | 2      | 4   | 6     | 8      | 10     | 12     |
|----------------------|--------|-----|-------|--------|--------|--------|
| F0B0                 | 9.88±2.14c | 20.54±2.69b | 21.74±2.14c | 21.94±1.76c | 22.88±2.03c | 25.17±2.33c |
| F1B0                 | 8.44±0.58bc | 19.08±0.70b | 19.47±1.00bc | 20.11±1.07bc | 21.10±1.24bc | 22.22±1.70bc |
| F1B1                 | 7.92±2.92bc | 18.61±3.06b | 18.83±3.00bc | 19.00±3.00bc | 20.18±3.04bc | 22.17±4.02bc |
| F1B2                 | 7.04±1.03abc | 18.90±1.15b | 19.00±1.09bc | 20.00±1.52b | 20.55±0.58bc | 21.98±1.00bc |
| F1B3                 | 6.84±0.63abc | 18.08±0.57b | 18.50±0.50bc | 19.22±0.50bc | 20.33±0.60bc | 21.36±1.04bc |
| F1B4                 | 6.28±1.93abc | 17.50±2.28ab | 17.83±1.04bc | 18.88±1.38bc | 19.78±1.41bc | 21.93±2.69bc |
| F1B5                 | 4.08±1.17a   | 13.13±2.06a  | 13.22±0.03a  | 13.55±1.43a  | 14.33±1.80a  | 15.03±1.41a  |
| F1B6                 | 7.92±1.27abc | 17.80±0.40ab | 18.44±0.91bc | 19.05±0.58bc | 19.25±0.50bc | 20.77±0.62bc |
| F2B0                 | 7.83±0.79bc | 17.55±1.45ab | 17.66±1.52bc | 18.22±1.50bc | 18.75±1.50bc | 19.36±1.84abc |
| F2B1                 | 5.94±1.77abc | 15.22±3.50ab | 16.17±4.04ab | 16.55±4.28ab | 16.90±4.25ab | 17.97±4.55ab |
| F2B2                 | 7.12±0.06abc | 17.16±3.23ab | 18.00±2.64bc | 19.11±1.01bc | 20.31±1.08bc | 21.56±2.03bc |
| F2B3                 | 6.22±3.53abc | 16.23±3.20ab | 17.76±1.18bc | 18.22±0.96bc | 19.21±0.76bc | 20.24±0.88bc |
| F2B4                 | 5.66±2.39abc | 17.51±1.45ab | 18.16±1.69bc | 18.22±1.07bc | 18.84±1.49bc | 19.96±1.44bc |
| F2B5                 | 6.70±0.30abc | 17.33±0.51ab | 17.83±0.92bc | 18.22±0.50bc | 19.05±0.25bc | 20.60±0.82bc |
| F2B6                 | 7.30±0.87abc | 16.75±1.43ab | 17.20±0.80bc | 17.33±0.88bc | 18.58±1.60bc | 20.23±2.28bc |

Remarks: Numbers (mean ± sd. n = 3) followed by different letters in the same column indicate significant differences, according to Duncan’s Multiple Range Test (DMRT) at the p<0.05 level.

Table 6. Periodic number of cocoa seeding leaves during the 12 weeks growth period after biostructure application.

| Biostructure sources | 2        | 4         | Week after biostructure application |
|----------------------|----------|-----------|-------------------------------------|
| F0B0                 | 2.77±0.69bc | 3.55±0.83a | 6.00±0.93b  | 7.66±0.88b | 7.77±1.07b | 9.55±1.57b |
| F1B0                 | 2.55±1.01bc | 3.88±0.38a | 5.66±0.33b | 6.88±0.50ab | 6.88±0.50ab | 7.88±2.12ab |
| F1B1                 | 1.44±1.50abc | 3.77±0.38a | 5.88±0.50b | 6.55±1.07ab | 7.33±0.88b | 7.77±1.64ab |
| F1B2                 | 1.00±0.00abc | 3.55±0.19a | 5.55±0.50b | 5.77±0.69ab | 6.77±1.34ab | 7.11±2.00ab |
| F1B3                 | 0.55±0.50a  | 3.66±0.33a | 5.33±0.57b | 6.44±0.69ab | 7.00±1.33ab | 8.11±2.69ab |
| F1B4                 | 0.88±0.83abc | 3.66±0.33a | 5.44±0.50b | 7.11±1.17b | 7.22±1.01b | 8.11±1.50ab |
| F1B5                 | 0.33±0.33a  | 2.88±1.64a | 3.88±2.03a | 4.88±2.91a | 5.00±2.88a | 5.22±3.15a |
| F1B6                 | 1.44±1.26abc | 4.00±0.33a | 5.66±0.88b | 6.66±0.66ab | 7.22±0.69b | 8.22±0.69ab |
| F2B0                 | 1.88±0.50abc | 3.88±0.69a | 5.22±0.69ab | 6.00±0.66ab | 5.55±0.69ab | 6.44±1.07ab |
| F2B1                 | 1.88±1.07abc | 3.22±0.83a | 4.88±1.01ab | 5.33±1.26ab | 5.33±0.57ab | 6.33±0.88ab |
| F2B2                 | 1.66±1.45abc | 3.66±0.00a | 4.66±0.88ab | 6.22±0.38ab | 6.44±0.38ab | 7.22±1.83ab |
| F2B3                 | 0.66±0.57abc | 3.55±0.83a | 5.33±0.66b | 6.44±0.83ab | 7.33±0.66b | 7.77±1.01ab |
| F2B4                 | 1.00±1.00abc | 3.77±0.19a | 5.88±0.38b | 6.22±0.69ab | 6.66±0.88ab | 7.33±1.20ab |
| F2B5                 | 1.22±0.69abc | 3.66±0.57a | 5.33±0.88b | 7.00±1.33b | 7.44±1.50b | 8.33±1.15ab |
| F2B6                 | 1.55±1.01abc | 3.44±0.69a | 5.44±0.50b | 6.00±0.66ab | 6.66±1.00ab | 8.00±1.85ab |

Remarks: Numbers (mean ± sd. n = 3) followed by different letters in the same column indicate significant differences, according to Duncan’s Multiple Range Test (DMRT) at the p<0.05 level.
The highest total AMF spore count in the biostructure was obtained using earthworm and ant populations in a 20:10 (individual/individual) ratio on G. sepium leaf and cocoa bean shell in a 50%-50% composition (Figure 3). This indicates the AMF spore density increased with increasing earthworm-ant ratio, confirming the presence of ants plays an important role in improving the soil pH, organic-C, N, and P contents (Almeida et al., 2019; Boots et al., 2012). Therefore, improved pH, organic-C, N, and P through bioturbation by ants, creates more suitable conditions for earthworm activity to form new biostructures (Sankar & Patnaik, 2018; Taylor et al., 2019). In addition, the biostructures’ environmental condition derived from the treatments modulates the growth of AMF spore populations (de Menezes et al., 2018; Lucas et al., 2017). The high density of AMF spores in the biostructure formed from a mixed composition of the organic matter types indicates the quality of food resources and the available conditions are suitable for collaboration between *Peryonix* sp. and *Dorylus* sp. In the present work, the addition of sago dregs to the organic matter mixture tended to decrease the spore density (Figure 3). Regarding the chemical attributes of the three organic matter types tested, sago dreg has a higher total-C and C/N ratio, but lower total-N, total-P, and total-K contents, compared to cocoa shell beans and *G. Sepium* leaves (Table 1). This is comparable with the report by Syaf et al. (2021), where the AMF spore density in the biostructure containing biochar decreased with the increasing earthworm population. Biochar is known to have high total-C and C/N and low nutrient content (Prasad et al., 2020), therefore, these results reaffirm that organic quality (total-C, C/N ratio, total-N, P, and K) has the capacity to affect AMF spore populations in soil biostructure created through activities of soil fauna ecosystem engineers (Medina-Sauza et al., 2019).

This study’s results discovered the total number of spores in rhizospheric soil from cocoa seedlings subjected to biostructure treatments, tend to be more, compared to seedlings subjected to soil applications without the ecosystem engineering soil fauna’s activities, while the percentage of infected roots differed insignificantly (Table 7). This indicates AMF spores carried by biostructures formed through the collaborative activity of ants and earthworms in soil from the smallholder cocoa plantation mixed with different types of organic matter, are able to germinate and produce hyphae infective to the cocoa seedlings’ roots. The highest number of spores in rhizospheric soil was obtained from the application of biostructures containing the highest amount of AMF spore. A study by (Harinikumar & Bagyara, 1994) also found earthworm casts and ant nests contained viable AMF spores infective to *Allium cepa* roots grown in sterilized soil. In this study, the inoculation of spores carried by the biostructure was performed under the conditions of cocoa seedlings grown on un-sterilized soil.

The large number of spores carried by the biostructure is the expected percentage of infected roots, but is also more, in some cases (Verbruggen et al., 2013). However, the results found another case, where the percentage of infected cocoa seedling roots differed insignificantly between treatments. This value ranged from 8.88% - 36.66% in all treatments, and was lower, compared to roots infected by arbuscular mycorrhizae from cocoa seedlings grown on sterilized soil (Aggangan et al., 2019). The success of AMF spore inoculation under unsterilized growing media is determined by biotic factors through an inhibitory mechanism (Fukami, 2015). The spore community inhabiting the growth media has the potential to suppress the performance of pores carried by inoculum source material to infect roots (Werner & Kiers, 2018).

**Table 7.** Spore AMF count, root infection and shoot dry weight of cocoa seedlings.

| Biostructure sources | AMF spore count (no. 50 g soil⁻¹) | Root infection (%) | Shoot dry weight (g) |
|----------------------|------------------------------------|--------------------|----------------------|
| F0B0                 | 16±4.70a                           | 16.66±17.64a       | 2.71±0.46a           |
| F1B0                 | 19±4.63a                           | 36.66±20.28a       | 2.14±0.09a           |
| F1B1                 | 30±5.56ab                          | 33.33±15.28a       | 2.58±0.52a           |
| F1B2                 | 33±9.89ab                          | 24.44±13.47a       | 1.91±0.37a           |
| F1B3                 | 26±9.33ab                          | 27.77±21.17a       | 1.88±0.46a           |
| F1B4                 | 15±7.58a                           | 21.11±25.24a       | 2.08±1.06a           |
| F1B5                 | 24±12.60ab                         | 30.00±18.56a       | 2.38±0.43a           |
| F1B6                 | 38±7.40b                           | 32.22±13.47a       | 2.42±0.61a           |
| F2B0                 | 23±5.34ab                          | 28.88±25.47a       | 1.88±0.45a           |
| F2B1                 | 23±7.21ab                          | 13.33±3.33a        | 2.24±0.55a           |
| F2B2                 | 28±12.72ab                         | 33.33±31.80a       | 1.86±0.92a           |
| F2B3                 | 32±7.18ab                          | 10.00±5.77a        | 2.09±0.53a           |
| F2B4                 | 32±12.25ab                         | 13.33±12.02a       | 1.77±0.22a           |
| F2B5                 | 28±7.50ab                          | 22.22±10.72a       | 1.86±0.81a           |
| F2B6                 | 29±11.57ab                         | 8.88±1.92a         | 2.85±1.44a           |

**Remarks:** Numbers (mean ± sd. n = 3) followed by different letters in the same column indicate significant differences according to Duncan’s Multiple Range Test (DMRT) at the p <0.05 level.
Furthermore, the inhibitory effect’s strength is supported by the nutrient availability in the growth media (Cely et al., 2016; Hayashi et al., 2018).

The percentage of infected roots did not differ significantly between the control and biostructures treatments. However, the plant height and the number of leaves were higher in the control, compared to the biostructure treatments (Table 5 and 6), but the seedling’s shoot dry weight was insignificant (Table 7). A study by Bagy Araji and Powell (1985) showed AMF application increased the height and stem diameter of marigolds grown in pots filled with unsterilized mineral soil with a characteristic pH and available P of 5.4 and 9µg/l, respectively. Meanwhile, Mau and Utami (2014) reported mycorrhizal spore inoculation did not show an increase in height for maize plants grown on un-sterilized soil dominated by sandy loam with a 7.02 pH. Also, Aggangan et al. (2019) reported the soil-based inoculant powder containing AMF spores increased the dry weight of inoculated cacao seedlings on non-sterilized soil enhanced with NPK fertilizer. In a report by Kim et al. (2017), inorganic fertilizer led to a higher increase in the dry weight of plants without spore inoculation, compared to counterparts inoculated with spores on sterile soil. Thus, this study’s results reaffirm the stoichiometry of soil ecology largely determining the mutualistic-parasitic symbiosis continuum in interactions between the spores and plant roots grown on soil under field conditions (Mandyam & Jumpponen, 2015).

5. CONCLUSION

The AMF spore density in the biostructure was affected by the composition of earthworms and ants, as well as the composition of organic matter mix added to the soil. In addition, the spore abundance was positively correlated with total, P, and negatively with the C/N ratio. The biostructure applied has the capacity to increase the total number of spores in the rhizospheric soil and roots infection from cacao seedlings growing on non-sterile soils. However, further studies are required to understand soil quality factors with the most contribution to a positive association between AMF spores carried through biostructure treatment.

Declaration of Competing Interest

The authors declare no competing financial or personal interests that may appear and influence the work reported in this paper.

References

Aggangan, N. S., Cortes, A. D., & Reaño, C. E. (2019). Growth response of cacao (Theobroma cacao L) plant as affected by bamboo biochar and arbuscular mycorrhizal fungi in sterilized and unsterilized soil. Biocatalysis and Agricultural Biotechnology, 22, 101347. https://doi.org/10.1016/j.bcab.2019.101347

Alimi, A., Adeleke, R., & Moteetee, A. (2021). Soil environmental factors shape the rhizosphere arbuscular mycorrhizal fungal communities in South African indigenous legumes (Fabaceae). Biodiversitas Journal of Biological Diversity, 22(5). https://doi.org/10.13057/biodiv/d220503

Almeida, A., Mitchell, A. L., Boland, M., Forster, S. C., Gloo, G. B., Tarkowska, A., Lawley, T. D., & Finn, R. D. (2019). A new genomic blueprint of the human gut microbiota. Nature, 568(7753), 499-504. https://doi.org/10.1038/s41586-019-0965-1

Asano, K., Kagong, W. V. A., Mohammad, S. M. B., Sakazaki, K., Talip, M. S. A., Sahmat, S. S., Chan, M. K. Y., Isoi, T., Kano-Nakata, M., & Ehara, H. (2021). Arbuscular Mycorrhizal Communities in the Roots of Sagor Palm in Mineral and Shallow Peat Soils. Agriculture, 11(11), 1161. https://doi.org/10.3390/agriculture11111161

Azizah Chulan, H. (1991). Effect of fertilizer and endomycorrhizal inoculum on growth and nutrient uptake of cacao (Theobroma cacao L) seedlings. Biology and Fertility of Soils, 11(4), 250-254. https://doi.org/10.1007/BF00335843

Bagy Araji, D. J., & Powell, C. L. (1985). Effect of vesicular-arbuscular mycorrhizal inoculation and fertiliser application on the growth of marigold. New Zealand Journal of Agricultural Research, 28(1), 169-173. https://doi.org/10.1080/00288233.1985.10427012

Bahrur, A., Fahimuddin, M., Safuan, L., Kilowasid, L. M., & Singh, R. (2018). Effects of cocoa pod husk biochar on growth of cacao seedlings in Southeast Sulawesi-Indonesia. Asian J. Crop Sci, 10(1), 22-33. https://doi.org/10.3923/ajcs.2018.22.30

Boots, B., Keith, A. M., Niechoj, R., Breen, J., Schmidt, O., & Clipson, N. (2012). Unique soil microbial assemblages associated with grassland ant species with different nesting and foraging strategies. Pedobiologia, 55(1), 33-40. https://doi.org/10.1016/j.pedobi.2011.10.004

Bottinelli, N., Jouquet, P., Capowiez, Y., Podwojewski, P., Grimaldi, M., & Peng, X. (2015). Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil and Tillage Research, 146, 118-124. https://doi.org/10.1016/j.still.2014.01.007

Briones, M. J. I. (2014). Soil fauna and soil functions: a jigsaw puzzle [Review]. Frontiers in Environmental Science, 2(7). https://doi.org/10.3389/fenvs.2014.00007

Cely, M. V. T., de Oliveira, A. G., de Freitas, V. F., de Luca, M. B., Barazzetti, A. R., dos Santos, I. M. O., Gionco, B., Garcia, G. V., Prete, C. E. C., & Andrade, G. (2016). Inoculant of Arbuscular Mycorrhizal Fungi (Rhizophagus clarus) Increase Yield of Soybean and Cotton under Field Conditions [Original Research]. Frontiers in Microbiology, 7(720). https://doi.org/10.3389/fmicb.2016.00720

Cobb, A. B., Wilson, G. W. T., Goad, C. L., & Grusak, M. A. (2018). Influence of alternative soil amendments on mycorrhizal fungi and cowpea production. Heliozyon, 4(7). https://doi.org/10.1016/j.heliozyon.2018.e00704

Cunha, L., Brown, G. G., Da Silva, E., Hansel, F. A., Jorge, G., McKey, D., Vidal-Torrado, P., Macedo, R. S., Velasquez, E., James, S. W., Lavelle, P., Kille, P., & Network, t. T. P. d. I. (2016). Soil Animals and Pedogenesis: The Role of Earthworms in

173
Anthropogenic Soils. *Soil Science*, 181(3/4), 110-125. https://doi.org/10.1097/SS.0000000000000144

de Menezes, A. B., Prendergast-Miller, M. T., Macdonald, L. M., Toscas, P., Baker, G., Farrell, M., Wark, T., Richardson, A. E., & Thrall, P. H. (2018). Earthworm-induced shifts in microbial diversity in soils with rare versus established invasive earthworm populations. *FEMS Microbiology Ecology*, 94(5), fiy051. https://doi.org/10.1093/femsec/fiy051

Dhar, P. P., & Mridha, M. A. U. (2012). Arbuscular mycorrhizal fungi on soybeans grown in long field with low phosphate availability. *Plant Nutrition*, 306-311. https://doi.org/10.1007/s11001-012-0241-9

Directorate General of Estates. (2019). *Tree Crop Estate Statistics of Indonesia 2018-2020: Cocoa*. Secretariat of Directorate General of Estates, Directorate General of Estates, Ministry of Agriculture. https://ditjenbun.pertanian.go.id/?publikasi-buku-publikasi-statistik-2018-2020

Ehrle, A., Potthast, K., Tischer, A., Trumbore, S. E., & Michalzik, B. (2019). Soil properties determine how Lasius flavus impact on topsoil organic matter and nutrient distribution in central Germany. *Applied Soil Ecology*, 133, 166-176. https://doi.org/10.1016/j.apsoil.2018.08.021

Forey, E., Chauvat, M., Coulibaly, S. F. M., Langlois, E., Barot, S., & Clause, J. (2018). Inoculation of an ecosystem engineer (Earthworm: Lumbricus terrestris) during experimental grassland restoration: Consequences for above and belowground soil compartments. *Applied Soil Ecology*, 125, 148-155. https://doi.org/10.1016/j.apsoil.2017.12.021

Franco, A. L. C., Cherubin, M. R., Cerri, C. E. P., Guimarães, R. M. L., & Cerri, C. C. (2017). Relating the visual soil structure status and the abundance of soil engineering invertebrates across land use change. *Soil and Tillage Research*, 173, 49-52. https://doi.org/10.1016/j.still.2016.08.016

Fukami, T. (2015). Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics*, 46, 1-23. https://doi.org/10.1146/annurev-ecolsys-110411-160340

Harinikumar, K. M., & Bagyaraj, D. J. (1994). Potential of earthworms, ants, millipedes, and termites for dissemination of vesicular-arbuscular mycorrhizal fungi in soil. *Biologia et Fertility of Soils*, 18(2), 115-118. https://doi.org/10.1007/BF00336456

Hayashi, M., Niwa, R., Urashima, Y., Suga, Y., Sato, S., Hirakawa, H., Yoshida, S., Ezawa, T., & Karasawa, T. (2018). Inoculum effect of arbuscular mycorrhizal fungi on soybeans grown in long-term bare-fallowed field with low phosphate availability. *Soil Science and Plant Nutrition*, 64(3), 306-311. https://doi.org/10.1007/s10380768.2018.1473007

INVAM. (2019). *Enumeration of Spores*. International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, West Virginia University. https://invam.wvu.edu/methods/spores/enumeration-of-spores

Jouquet, P., Janeau, J.-L., Pisano, A., Sy, H. T., Orange, D., Minh, L. T. N., & Valentin, C. (2012). Influence of earthworms and termites on runoff and erosion in a tropical steep slope fallow in Vietnam: A rainfall simulation experiment. *Applied Soil Ecology*, 61, 161-168. https://doi.org/10.1016/j.apsoil.2012.04.004

Kameoka, H., Tsutsui, I., Saito, K., Kikuchi, Y., Handa, Y., Ezawa, T., Hayashi, H., Kawaguchi, M., & Akiyama, K. (2019). Stimulation of asymbiotic sporulation in arbuscular mycorrhizal fungi by fatty acids. *Nature Microbiology*, 10(16), 1654-1660. https://doi.org/10.1038/s41564-019-0485-7

Kilowasid et al. *SAINS TANAH – Journal of Soil Science and Agroclimatology*, 18(2), 2021

Lee, K. K., Reddy, M. V., Wani, S. P., & Trimurtulu, N. (1996). Vesicular-arbuscular mycorrhizal fungi in earthworm casts and surrounding soil in relation to soil management of a semi-arid tropical Alfisol. *Applied Soil Ecology*, 3(2), 177-181. https://doi.org/10.1016/0929-1393(95)00082-8

Lucas, J., Bill, B., Stevenson, B., & Kaspari, M. (2017). The microbiome of the ant-built home: the microbial communities of a tropical arboreal ant and its nest. *Ecosphere*, 8(2), e01639. https://doi.org/10.1002/ecs2.1639

Mandiyam, K. G., & Jumpponen, A. (2015). Mutualism–parasitism paradigm synthesized from results of root-endophyte models [Hypothesis and Theory]. *Frontiers in Microbiology*, 5(776). https://doi.org/10.3389/fmicb.2014.00776

Mau, A. E., & Utami, S. R. (2014). Effects of biochar amendment and arbuscular mycorrhizal fungi inoculation on availability of soil phosphorus and growth of maize [arbuscular mycorrhizal fungi; biochar; calcareous soil; maize; phosphorus uptake]. 2014, 1(2), 6. https://doi.org/10.15243/jdoml.2014.012.069

Medina-Saaua, R. M., Álvarez-Jíménez, M., Delhal, A., Reverchon, F., Blouin, M., Guerrero-Analco, J. A., Cerdán, C. R., Guevara, R., Villain, L., & Barois, I. (2019). Earthworms Building Up Soil Microbiota, a Review [Systematic Review]. *Frontiers in Environmental Engineering*. 174
Sains Tanah – Journal of Soil Science and Agroclimatology, 18(2), 2021

Mei, L., Yang, X., Cao, H., Zhang, T., & Guo, J. (2019). Arbuscular Mycorrhizal Fungi Alter Plant and Soil C:N:P Stoichiometries Under Warming and Nitrogen Input in a Semiarid Meadow of China. *International Journal of Environmental Research and Public Health*, 16(3), 397. https://doi.org/10.3390/ijerph16030397

Melo, C. D., Walker, C., Krüger, C., Borges, P. A. V., Luna, S., Mendonça, D., Fonseca, H. M. A. C., & Machado, A. C. (2019). Environmental factors driving arbuscular mycorrhizal fungal communities associated with endemic woody plant Picconiaazoricana on native forest of Azores. *Annals of Microbiology*, 69(13), 1309-1327. https://doi.org/10.1007/s13213-019-01535-x

Moreira, S. D., França, A. C., Rocha, W. W., Tibães, E. S., & Neiva, E. (2018). Inoculation with mycorrhizal fungi on the growth and tolerance to water deficit of coffee plants. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 22, 747-752. https://doi.org/10.1590/1807-1929/agriambi.v22n11p747-752

Powell, C. L., & Bagyaraj, D. J. (2017). *Va Mycorrhizae: Why all the Interest?* In C. L. Powell & D. J. Bagyaraj (Eds.), *VA Mycorrhiza*. CRC Press. https://doi.org/10.1201/9781351077514

Prasad, M., Chrysargyris, A., McDaniel, N., Kavanagh, A., Gruda, N. S., & Tzortzakis, N. (2020). Plant Nutrient Availability and pH of Biochars and Their Fractions, with the Possible Use as a Component in a Growing Media. *Agronomy*, 10(1), 10. https://doi.org/10.3390/agronomy10010010

Rillig, M. C., Aguilar-Trigueros, C. A., Anderson, I. C., Antonovics, J., Ballhausen, M.-B., Bergmann, J., Bielcik, M., Chaudhary, V. B., Deveautour, C., Grünfeld, L., Hempel, S., Lakovic, M., Lammel, D. R., Lehmann, A., Lehmann, J., Leifheit, E. F., Liang, Y., Li, E., Lozano, Y. M., Manntschke, A., Mansour, I., Oviatt, P., Pinek, L., Powell, J. R., Roy, J., Ryo, M., Sosa-Hernández, M. A., Veresoglou, S. D., Wang, D., Yang, G., & Zhang, H. (2020). Myristate and the ecology of AM fungi: significance, opportunities, applications and challenges. *New Phytologist*, 227(6), 1610-1614. https://doi.org/10.1111/nph.16527

Salim, M. A., Budi, S. W., Setyaningsih, L., Iskandar, Wahyudi, I., & Kimi, H. (2020). Root colonization by arbuscular mycorrhizal fungi (AMF) in various age classes of revegetation post-coal mine. *Biodiversitas Journal of Biological Diversity*, 21(11). https://doi.org/10.13057/biodiv/d211105

Sanjaya, M. F., Kilowasid, L. M. H., Sabaruddin, L., Sulaeman, D., & Nurmas, A. (2020). Pengaruh Bahan Organik terhadap Spora Fungi Mikoriza Arbuskula dalam Tanah, dan Potensi Tanahnya Sebagai Sumber Inokulum. *Berkala Penelitian Agronomi*, 8(1), 11-22. http://oj.s.uho.ac.id/index.php/agnroma/article/view/12938

Sankar, A. S., & Patnaik, A. (2018). Impact of soil physicochemical properties on distribution of earthworm populations across different land use patterns in southern India. *The Journal of Basic and Applied Zoology*, 79(1), 50. https://doi.org/10.1186/s41936-018-0066-y

Schultz, T. R., Sosa-Calvo, J., Brady, S. G., Lopes, C. T., Mueller, U. G., Bacci Jr., M., & Vasconcelos, H. L. (2015). The Most Relictual Fungus-Farming Ant Species Cultivates the Most Recently Evolved and Highly Domesticated Fungal Symbiont Species. *The American Naturalist*, 185(5), 693-703. https://doi.org/10.1086/680501

Seutra Kaba, J., Abunyewa, A. A., Kugbe, J., Kwashie, G. K. S., Owusu Ansah, E., & Andoh, H. (2021). Arbuscular mycorrhizal fungi and potassium fertilizer as plant biostimulants and alternative research for enhancing plants adaptation to drought stress: Opportunities for enhancing drought tolerance in cocoa (Theobroma cacao L.). *Sustainable Environment*, 7(1), 1963927. https://doi.org/10.1080/27658511.2021.1963927

Shukla, R. K., Singh, H., & Rastogi, N. (2016). How effective are disturbance – tolerant, agroecosystem – nesting ant species in improving soil fertility and crop yield? *Applied Soil Ecology*, 108, 156-164. https://doi.org/10.1016/j.apsi.2016.08.013

Sivakumar, N. (2013). Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. *Annals of Microbiology*, 63(1), 151-160. https://doi.org/10.1007/s13213-012-0455-2

Sugiura, Y., Akiyama, R., Tanaka, S., Yano, K., Kameoka, H., Marui, S., Saito, M., Kawaguchi, M., Akiyama, K., & Saito, K. (2020). Myristate can be used as a carbon and energy source for the asymbiotic growth of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences*, 117(41), 25779. https://doi.org/10.1073/pnas.2006948117

Syaf, H., Pattah, M. A., & Kilowasid, L. M. H. (2021). Quality of soil from the nickel mining area of Southeast Sulawesi, Indonesia, engineered using earthworms (Pheretima sp.) [biostreut; ecosystem engineer; soil ecological engineering; soil quality]. 2021, 8(4), 11. https://doi.org/10.15243/jdmlm.2021.084.2995

Taylor, A. R., Lenoir, L., Vegerfors, B., & Persson, T. (2019). Ant and Earthworm Bioturbation in Cold-Temperate Ecosystems. *Ecosystems*, 22(5), 981-994. https://doi.org/10.1007/s10021-018-0317-2

Verbruggen, E., van der Heijden, M. G. A., Rillig, M. C., & Kiers, E. T. (2013). Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist*, 197(4), 1104-1109. https://doi.org/10.1111/j.1469-8137.2012.04348.x

Verzeaux, J., Nivelle, E., Roger, D., Hirié, B., Dubois, F., & Tetu, T. (2017). Spore Density of Arbuscular Mycorrhizal Fungi is Fostered by Six Years of a No-Till System and is Correlated with Environmental Parameters in a Silty Loam Soil. *Agronomy*, 7(2), 38. https://doi.org/10.3390/agronomy7020038

Vogt, D. J., Tilley, J. P., & Edmonds, R. L. (2015). *Soil and Plant Analysis for Forest Ecosystem Characterization*. Berlin, Germany: Springer-Verlag.
München, Boston: De Gruyter. 
https://doi.org/10.1515/9783110290479

Wang, P., Wang, Y., Shu, B., Liu, J.-F., & Xia, R.-X. (2015). Relationships Between Arbuscular Mycorrhizal Symbiosis and Soil Fertility Factors in Citrus Orchards Along an Altitudinal Gradient. *Pedosphere, 25*(1), 160-168. https://doi.org/10.1016/S1002-0160(14)60086-2

Wang, S., Li, J., Zhang, Z., Chen, M., Li, S., & Cao, R. (2019). Feeding-strategy effect of Pheidole ants on microbial carbon and physicochemical properties in tropical forest soils. *Applied Soil Ecology, 133*, 177-185. https://doi.org/10.1016/j.apsoil.2018.10.006

Werner, G. D. A., & Kiers, E. T. (2015). Order of arrival structures arbuscular mycorrhizal colonization of plants. *New Phytologist, 205*(4), 1515-1524. https://doi.org/10.1111/nph.13092

Wills, B. D., & Landis, D. A. (2018). The role of ants in north temperate grasslands: a review. *Oecologia, 186*(2), 323-338. https://doi.org/10.1007/s00442-017-4007-0

Zanella, A., Ponge, J.-F., & Briones, M. J. I. (2018). Humusica 1, article 8: Terrestrial humus systems and forms – Biological activity and soil aggregates, space-time dynamics. *Applied Soil Ecology, 122*, 103-137. https://doi.org/10.1016/j.apsoil.2017.07.020

Zanella, A., Ponge, J.-F., Topoliantz, S., Bernier, N., & Juilleret, J. (2018). Humusica 2, Article 15: Agro humus systems and forms. *Applied Soil Ecology, 122*, 204-219. https://doi.org/10.1016/j.apsoil.2017.10.011