EFFECT OF THE SOIL PROTECTION SHEET AND THE BAG MATERIAL ON THE SOIL MICROORGANISMS AND THE INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI IN THE VOLCANIC DEVASTATED SITE IN MT. BATUR, BALI, INDONESIA

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ABSTRACT

In the volcanic devastated site of Mt. Batur located in the north eastern part of Bali, Indonesia, the test construction with the soil protection sheet and the bag material for the prevention of soil erosion and the revegetation had been executed in December, 2012 and the effect has been verified by now. In this study, the effect of the sheet on the soil microorganisms was investigated and the effect of the indigenous arbuscular mycorrhizal fungi, which had been put into the bag material with 3 kinds of woody plant seeds, on the vegetation was verified. As a result, after 2 years and 8 months from the construction, it was showed that the soil microorganisms under the sheet without the plant increased 2 to 2.4 times more than those in the bare site without the plant. Furthermore, those soil microorganisms under the sheet with the plant increased 8.4 to 8.9 times more than those in the bare site without the plant. It is considered that the cutting fragments in the length of about 5cm of the gramineous plant root existed near the construction site put into the bag material with the woody plant seeds contributed to the colonization of the arbuscular mycorrhizal fungi into the roots of seedlings from the seeds and the survival of the seedlings from the seeds as the inocula of the fungi.

Keyword: Revegetation, mycorrhizal fungi, protection sheet

INTRODUCTION

In the surrounding of Mt. Batur located in the north eastern part of Bali, Indonesia, there are the volcanic deposits such as the volcanic ash, scoria, volcanic rock and the weathered gravel and the sand etc. derived from the eruption in 1917 and 1926. The deposits are easy to be eroded by the rainfall etc. even in present when about 90 years have passed since the eruption and the establishment and the growth of plants is difficult because those are short of nutrients for plants. And the muddy water and the deposits are flown into the Lake Batur by the rainfall etc., so that the deterioration of water quality is concerned. The forest area decreases by the deforestation, so that the effect on the local or the earth environment is also concerned. Therefore, the measure to prevent the erosion of deposits by the rainfall etc. is needed. By now, in this site, the planting of seedlings has been executed as the measure. Although the revegetation proceeds little by little by the measure, it is not enough to be revegetate. In the volcanic devastated site such as Mt. Unzen-Fugen dake in Nagasaki Prefecture and Mt. Sakurajima in Kagoshima Prefecture in Japan, it is shown that the soil protection sheet, which is effective to the prevention of soil erosion, and the bag material, which enables the vegetation from the woody plant seeds, are effective to the prevention of soil erosion and the revegetation[1)]. Therefore, in the similar site in Indonesia, the verification of the effect of the application of the sheet and the bag material on the prevention of soil erosion and the revegetation and the suggestion as one of the effective construction method are aimed. By now, it is reported that the soil protection sheet prevented the soil erosion even after 2 years and 3 months from the construction and made the vegetation coverage rate improve and the ratio of the bag material from which the woody plant seedlings grew from the plant seeds put into the bags to the total number of bag materials was 32.8%[2)]. In this paper, the effect of the soil protection sheet on the amount of soil microorganisms and the verification of the effect of the indigenous arbuscular mycorrhizal fungi put into the bag materials on the vegetation are aimed. The report content is one executed in the Pilot Survey for Disseminating SME's Technologies for Disaster Prevention and Environmental Regeneration that is commissioned projects of Japan International Cooperation Agency (JICA).

MATERIALS AND METHODS

Outline of the experimental site

The test construction was executed in the below 2 sites of Mt. Batur, Kintamani district, Bangli regency, Bali, Indonesia (Fig.1).

Fig.1. Location of the experimental sites

1) A site

In December, 2012, the soil protection sheet, of which product name is Takino Filter Inc., was covered in the area of 2,000m^2 in the gentle slope of the under part in the east-southeast direction of Mt. Batur. In this site, the underground is composed of the scoria, the volcanic rock and the weathered gravel and sand etc. and there are comparatively many gramineous grass plants and a few leguminous shrubs and tall woody plants such as Sumatran pine. The used soil protection sheet is composed of 2 layers which the non-woven fabric of 60g/m^2 is reinforced by the net (Picture 1a). The sheet has the porosity of 97 to 98% and the ultrafine fiber of the non-woven fabric entangles the surface soil grains and adheres to the soil surface and so that the soil erosion is prevented effectively. And the sheet has the functions of water holding and the decrease of the change of underground temperature such as the suppression of rise of it in summer and the prevention of the drop of it in winter etc. And the sheet enables the penetration of the bud and the root of plants and the revegetation. In present, 2 years and 8 months have passed since the construction, but the soil erosion is prevented effectively and the vegetation recovers very well as the report[2)] (Fig. 3a).

2) B site

In December, 2012, the soil protection sheet was covered in the area of 500m^2 in the flat, the gentle slope and the steep slope of the under part in the southwest direction of the Mt. Batur and then 67 bag materials were put on the sheet in the intervals of a few meters at random. The same sheet described above the A site was used. The bag material, which the volcanic deposits of the amount of 1 to 2 litters mixed with the gramineous root fragments cut into about 5 cm length was put into the 2 kinds of bags made of the polyethylene net with the paper or the palm net with the polyester non-woven fabric with 3 kinds of woody plant seeds, was used (Fig. 2b, 2c). The kind and the number of used woody plant seeds were 20 seeds for Teak, 150 seeds for *Leucaena leucocephala* and 5 seeds for Jack fruit. In present, 2 years and 8 months have passed since the construction, but the ratio of the bag materials in which the alive seedlings remain to the total number of bags is comparatively high of 32.8% and Teak and *Leucaena leucocephala* grow well particularly as the report (Fig. 3b)[2)].

Collection of the samples

1) A site

For the investigation of the amount of microorganisms, the samples of volcanic deposits were collected in 7, August, 2015. Total 8 samples were collected from each 2 points of non-rhizosphere or rhizosphere part in the bare plot and the sheet covered plot. The surface part to 5 to 10 cm depths was collected. In this time, the underground temperature in each sampling plot was measured with the stick thermometer. The collected samples were put into the plastic bags and brought back to the laboratory and kept in intact condition under the room temperature until the investigation.

2) B site

For the investigation of the spore number and the colonization condition into plant roots by AM fungi, each bag material with the polyethylene net or the palm net from which the gramineous grass plants grow and the deposits just under of the bag and the sheet and the plant with roots and the rhizosphere deposits of 2 gramineous grass plants and the non-rhizosphere deposits from 2 points in the bare plot were collected in 7, August, 2015. The collected samples were put into the plastic bags and brought back to the laboratory and kept in intact condition under the room temperature until the investigation as same as the above A site.

Fig. 2. Appearance of the soil protection sheet and the bag material

(a)Soil protection sheet (W 1m, L50m) (b)Bag material with the polyethylene net (300mm×430mm) (c)Bag material with the palm net (300mm×430mm)

Fig. 3. Condition of each test construction site after 2 years and 8 months from the construction (7, August, 2015)

(a)A site (b)B site

Number of microorganisms

In the volcanic deposit samples collected in the test construction A site, the number of bacteria and actinomycetes and fungi was investigated by the dilution plate method. YG agar medium (yeast extract 1.0g, D-glucose 1.0g, dipotassium hydrogen phosphate 0.3g, potassium dihydrogen phosphate 0.2g, magnesium sulfate heptahydrate 0.2g, agar 15.0g, cycloheximide 0.050g, distilled water 1,000mL) for the bacteria and the actinomycetes and Rose Bengal agar medium (D-glucose 10.0g, peptone 5.0g, rose bengal 0.033g, potassium dihydrogen phosphate 1.0g, magnesium sulfate heptahydrate 0.5g, agar 20.0g, streptomycin sulfate 0.030g, distilled water 1,000mL) for the fungi were used respectively. The number of colony which occurred after one week from smearing the diluted sample on the agar media was counted. In this time, the water content was also measured and then the number of microorganisms per dry matter was calculated.

Spore density of AM fungi

In the volcanic deposit samples collected in the test construction B site, each sample was weighed by 25g in the Erlenmyer flask of 100mL and then the supernatant of the suspension was sieved in about 5 times repeatedly by the wet sieving method. The remnant on each sieve of 53µm, 106µm and 212µm were collected in the centrifuge tube and those were centrifuged at 5,000 rpm for 3 minutes. The supernatant after the centrifugation was transferred into each Petri dish and then the spore number of AM fungi was counted under the stereomicroscope. When the quantity of remnant or the spore number was too much to count, the sample was diluted in 10 to 100 times and then the spore number was counted. In this case, the water content was also measured and then the spore density per dry matter was calculated.

Colonization into the plant roots by AM fungi

Among the samples collected in the test construction B site, each gramineous grass plant roots were cut into 3 to 5 cm randomly and put them in the beaker of 100mL. The plant roots were washed in the tap water and the volcanic deposits adhered to the plant roots were dropped out. The cleaned plant roots were stained by the trypan blue and some stained roots were arranged in the Petri dish and some ones were prepared as the specimen. With the former prepared samples, the colonization rate into plant roots by AM fungi was measured by the grid line intersection method under the stereomicroscope and with the latter prepared specimens, the detail structures of AM fungi in plant roots were observed under the biological microscope.

RESULTS AND DISCUSSIONS

Number of microorganisms

The number of bacteria and actinomycetes in the rhizosphere part of the soil protection sheet covered plot and that in the non-rhizosphere part of the bare plot were 3.5×16^{6}CFU/g dry matter and 4.1×10^{5}CFU/g dry matter on an average respectively, so that the former was 8.4 times more than the latter (Figure 2). And that in the non-rhizosphere part of the soil protection sheet covered plot and that in the rhizosphere part of the bare plot were 1.0×10^{6}CFU/g dry matter and 7.1×10^{5}CFU/g dry matter on an average respectively, so that the former was 2.4 times and the latter was 1.7 times more than that in the non-rhizosphere part of the bare plot (Fig. 4). However, there was no difference among every plot statistically by the Tukey's range test. The number of fungi in the rhizosphere part of the sheet covered plot and that in the non-rhizosphere part of the bare plot were 2.3×16^{5}CFU/g dry matter and 2.5×10^{4}CFU/g dry matter on an average respectively, so that the former was 8.9 times more than the latter (Fig. 5). And that in the non-rhizosphere part of the sheet covered plot and that in the rhizosphere part of the bare plot were 5.0×10^{4}CFU/g dry matter and 3.5×10^{4}CFU/g dry matter on an average respectively, so that the former was

2.0 times and the latter was 1.4 times more than that in the non-rhizosphere part of the bare plot (Fig. 5). However, there was also no difference among every plot statistically by the Tukey's range test.

From the above results, it was showed that once the plants establish and grow on the bare site, microorganisms in the plot increase by 1.4 to 1.7 times more of that in the bare plot where the there is no plants but the coverage with the soil protection sheet makes microorganisms increase by 2.0 to 2.4 times more of it after 2 years and 8 month from the construction. This shows that in this construction site, the mulching effect by the soil protection sheet is more effective than the rhizosphere effect by the plants for the increase of microorganisms in the volcanic deposits. Furthermore, when the plants established and grew on the soil protection sheet, microorganisms in the plot increased by 8.4 to 8.9 times of that in the bare plot without the plants, so that it was showed that the combination of the mulching effect by the soil protection sheet and the rhizosphere effect by the establishment and the growth of plants makes microorganisms increase remarkably. By now, according to the result which the amount of soil erosion was investigated with the soil protection sheet, it was showed that the soil erosion was suppressed by the sheet only but the increase of plant root density by the establishment and the growth of plants makes the effect increase much remarkably[3], so that it is considered that this leads to the remarkable increase of microorganisms in the volcanic deposits. In the comparison of temperature in the surface of volcanic deposits measured in the sampling daytime, that in the rhizosphere part of the soil protection sheet covered plot was 26.6℃ on an average, so that it was lower by 6.2℃ than that in the non-rhizosphere part of the bare plot of 32.8℃ on an average (Figure 6). The temperatures in the non-rhizosphere part of the sheet covered plot and that in the rhizosphere part of the bare plot were 31.9℃ and 31.1℃ on an average respectively, so that those were lower by 0.9 ℃ and 1.7 ℃ than it (Fig. 6). This shows that the mulching by the soil protection sheet make the increase of temperature in the hot time of daytime suppress and the shadow by plants is also effective to the decrease of temperature and the combination of the mulching effect by the sheet and the shadow effect by plants makes the rise of temperature suppress more effectively and so supplies the moderate condition of temperature not only for the growth of plant roots but also the living of microorganisms in the site.

From above results, it is considered that the coverage of the soil protection sheet itself makes microorganisms in the volcanic deposits increase by the soil erosion prevention effect and the suppression effect of the rise of temperature in the daytime etc., and once the plants establish and grow on the sheet, the effect becomes more remarkably by satisfying the condition capable of the increase of microorganisms. Furthermore, it is considered that the increase of microorganisms contributes to the formation of water stable soil aggregates because the adhesive materials by microorganisms themselves and the secretion and fungal hyphae entangle soil particles. Thus, it is expected that the soil physical condition is improved and more moderate soil for the plant growth is formed by the soil protection sheet.

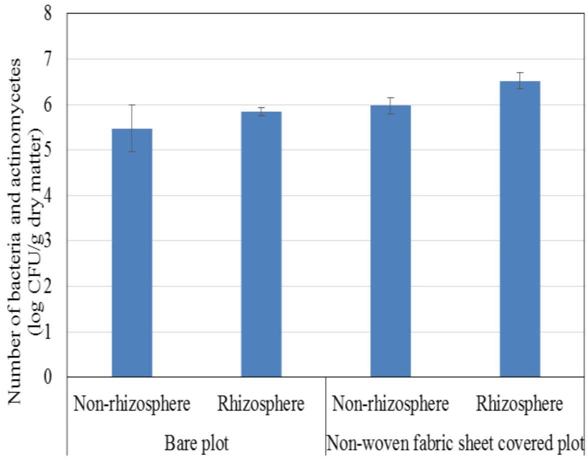

Fig. 4. Effect of the soil protection sheet and the plant on the number of bacteria and actinomycetes in the A site of the test construction site of Mt. Batur, Bali, Indonesia after 2 years and 8 months from the construction (7 August, 2015).

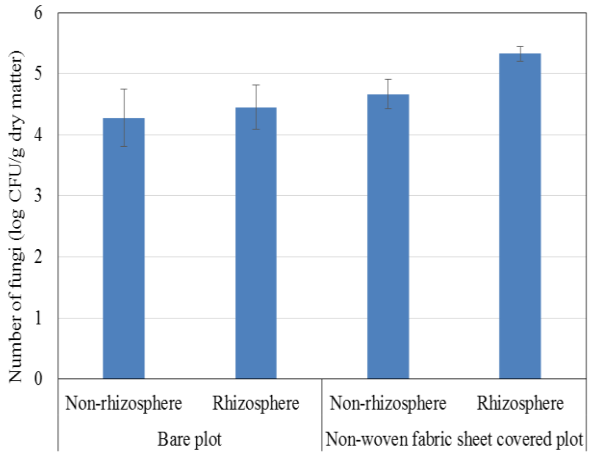

Fig. 5. Effect of the soil protection sheet and the plant on the number of fungi in the A site of the test construction site of Mt. Batur, Bali, Indonesia after 2 years and 8 months from the construction (7 August, 2015).

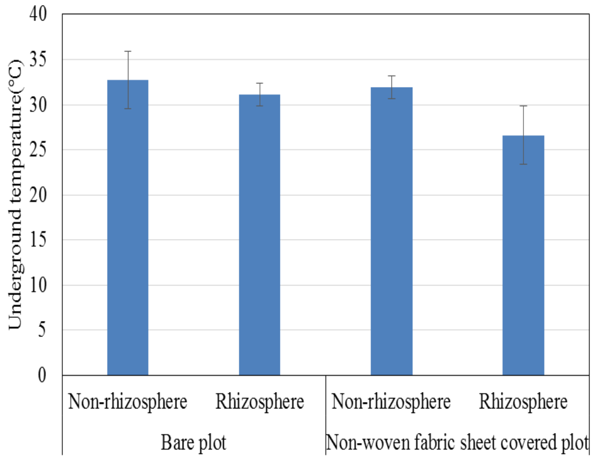

Fig. 6. Effect of the soil protection sheet and plants on the underground temperature in the daytime in the A site of test construction site of Mt. Batur, Bali, Indonesia after 2 years and 8 months from the construction (7, August, 2015).

Spore density of AM fungi

In the test construction B site of Mt. Batur, the spore density of AM fungi in the surface part of the bare plot where the plants does not exist was 8 spores/g dry matter on an average, but that inside the bag material was 60 spores/g dry matter and that just under the bag and the soil protection sheet was 39 spores/g dry matter on an average and so those were 7.1 times and 4.6 times more than that in the bare site without plants (Table 1). While, that in the rhizosphere part of the gramineous grass plants in the bare plot was 87 spores/g dry matter on an average and so that was 10.3 times more than that in the bare plot without plant (Table 1). Particularly, that in the rhizosphere part of the gramineous grass plant which grows in the flat and sandy ground about 10m far from the soil protection sheet covered plot was 137 spores/g dry matter (Table 1). This shows that in the test construction site, there are a large number of spores of AM fungi adapted to the site in the rhizosphere part of the gramineous grass plants which grow in the flat and good conditioned plot. And in the site, even if it is the bare site without plants, in the surrounding part the plants establish and grow, so that it is considered that there are a few spores of AM fungi affected by the existence of the plants.

Colonization into the plant roots by AM fungi

The colonization rate into the plant roots by AM fungi in the gramineous grass plants in the bare plot was 90.2% in average of two plants, that in the gramineous grass plants inside the bag material was 92.1% in average of two plants and that in the same plants just under of the bag and the soil protection sheet was 80.2% in average of two plants, so that it is showed that every plant roots are colonized extreme highly by AM fungi although that just under the bag and the sheet is lower by about 12% than that inside the bag (Table 1). This suggests that the indigenous AM fungi put into the bag material as the fragmented gramineous grass plant roots colonized by the fungi contribute largely to the establishment and the growth of plants in the site accompanied with the high spore density of AM fungi in the volcanic deposits.

Concerning the structures of AM fungi in the plant roots, the inner hyphae and the vesicle in every plant roots were observed (Fig 7). In almost plant roots, the inner spores were also observed, but in the gramineous grass plant roots inside the bag material with the palm net and just under the bag and the sheet, the inner spores with the comparative big size of 70 to 120μm were observed particularly (Fig 7e and 7f).

Effect of the application of the indigenous AM fungi

This time, in the seedling planting plot in the neighbor of the test construction B site of Mt. Batur, the number of survived seedlings was 28 in the total number of 122 counted and so the survival rate was 23.0%. While, the survival rate of the bag material in which the seedlings from the seeds put into the bag survived and grew after 2 years and 3 months from the construction was 32.8%, so that it was about 10% higher than that in the seedling planting method. This shows that in the rhizosphere part of the gramineous grass plants dotted in the test construction B site of Mt. Batur, the spore of the indigenous AM fungi adapted to the site exists in extremely high dense in present when about 90 years have passed since the eruption, in the bare site without plants, putting them into the bag material makes the indigenous AM fungi colonize into the seedling roots from the seeds put into the bag together and contributes to the improvement of the survival.

Table 1. Spore density of AM fungi and the colonization into the plant roots by the fungi in the test construction B site of Mt. Batur after 2 years and 8 months from the construction (7, August, 2015)

Investigation plot	Sampling plot	Sampling part	Spore density of AM fungi（spores/g dry matter）	Colonization rate（%）	Structure of AM fungi in the plant roots
Bare plot	beside the soil protection sheet	Surface part without plants	7	ー	ー
		Rhizosphere part in the gramineous grass plant	36	86.2	Inner hyphae, vesicle, inner spore
	Plot about 10 m far from the soil protection sheet covered plot	Surface part without plants	10	ー	ー
		Rhizosphere part in the gramineous grass plant	137	94.2	Inner hyphae, vesicle
Bag material＋soil protection sheet covered plot	Bag material with the polyethylene net	Inside the bag material	37	98.4	Inner hyphae, vesicle, inner spore
		Just under the bag material and the soil protection shee	43	93.0	Inner hyphae, vesicle, inner spore
	Bag material with the palm net	Inside the bag material	83	85.9	Inner hyphae, vesicle, inner spore
		Just under the bag material and the soil protection shee	35	67.3	Inner hyphae, vesicle, inner spore

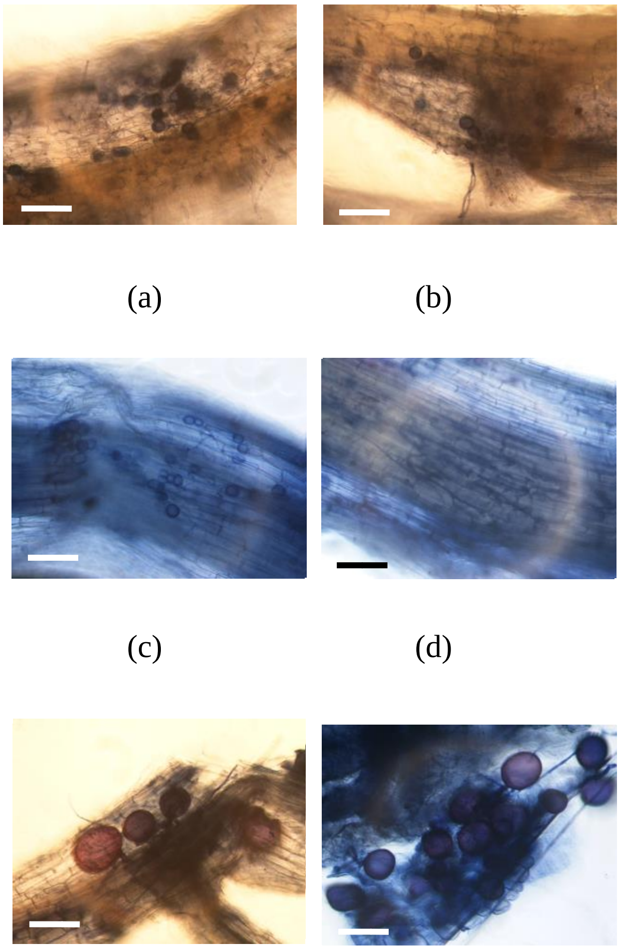

Fig. 7. Colonization condition into each plant roots by AM fungi (Bar length in the left under: 100µm). with the palm net and the soil protection sheet.

(a) Gramineous grass plant roots in the bare plot beside the soil protection sheet covered plot,
(b) Gramineous grass plant roots in the bare plot about 10m far from the soil protection sheet covered plot.
(c) Gramineous grass plant roots inside the bag material with the polyethylene net,
(d) Gramineous grass plant roots just under the bag material with the polyethylene net and the soil protection sheet.
(e) Gramineous grass plant roots inside the bag material with the palm net,
(f) Gramineous grass plant roots just under the bag material

CONCLUSION

This time, in the two test construction sites of Mt. Batur, the effect of the soil protection sheet on the microorganisms in the A site and the verification of the effect of the indigenous AM fungi put into the bag material were investigated in the B site and the following results were obtained.

(1) It was showed that the soil protection sheet itself contributes to the increase of microorganisms in the A site, but furthermore, the establishment and the growth of the local plants on the sheet make the effect increase more remarkably.
(2) In present when about 90 years have passed since the eruption in the volcanic devastated site of Mt. Batur, in the rhizosphere part of dotted gramineous grass plants, a large number of spores of AM fungi exist, so that it is considered that putting them into the bag material with the woody plant seeds as the inocula of indigenous AM fungi makes the fungi colonize into the woody seedlings from the seeds put into the bag and contributes to the improvement of the survival.

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