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Terrestrial Arthropod Profile and Soil Microbial Population Dynamics on Cabbage Cropping as Affected by Application of *Trichoderma* Microbial Inoculant (TMI) in Quezon, Sariaya, Philippines

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ABSTRACT

A two-season study was conducted in a cabbage farmer's field in Sariaya, Quezon Province, Philippines to determine the impact of Trichoderma microbial inoculant (TMI) application on above-ground terrestrial arthropod profile. Soil microflora population dynamics, soil chemical properties, the nitrogen and phosphate crop uptake, and disease incidence were monitored for the first season only. TMI treatment was compared with farmer's practice (FP, control) that involved insecticide treatment following an RCBD with three replicates. Target organisms were simultaneously monitored at 40, 60 and 85 days after transplanting (DAT). This is the first report on simultaneously studying these components of the cabbage agroecosystem.

Combined seasons' data showed higher arthropod counts in TMI plots, with predators significantly higher at third sampling-first season and parasite population first sampling-second season. Insect damage was observed on all plants indicating herbivore infestation, despite insecticide spraying in FP. Marketable yield was significantly greater in TMI plots based on combined seasons' data.

Trichoderma invaded cabbage roots and existed as an endophyte throughout the life of the crop. It also significantly reduced disease incidence, increased N uptake despite reduced fertilizer application compared with FP. No change in culturable bacterial and fungal population was observed except for a transient increase in fungal population following TMI application.

Further testing on other crucifers should be done to determine their reaction against major insect pests and on functional microbial groups.

Key words: *Trichoderma microbial inoculant, arthropod profile, cabbage, bacterial and fungal population dynamics*

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INTRODUCTION

Species of *Trichoderma* belong to group of beneficial fungi. They are free-living and common in soil and root ecosystems. Some species and strains are highly interactive in root, soil and foliar environments and produce many different enzymes able to degrade a broad range of organic molecules such as cellulose and chitin, and known for their antagonistic abilities toward many plant pathogenic fungi (*Harman and Bjorkman 1998; Papavizas 1985*). The highly reactive strains improve growth of host crops; enhance their survival from infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion (*Harman 2006*). Studies on the antagonistic modes of action and on the production of secondary metabolites have been numerous (*Tronsmo et al. 1993; Harman 2006*). Furthermore, there are also studies on the impact of *Trichoderma* on other beneficial microbes like arbuscular mycorrhiza, ectomycorrhiza and *Rhizobium* (*Brimmer and Boland 2003*), but few researches deal with the impact of the *Trichoderma* on non-target microbes, i.e. the soil microbial community.

In the Philippines, *Trichoderma* has become popular since the endorsement by the government of *T. harzianum* as activator for compost used for crop production. Earlier known as the IBS Rapid Composting Technology, popularized by its inventor Dr. Virginia C. Cuevas, the *Trichoderma* technology was transferred to the Filipino farmers in 1990 through funds provided by Philippine Council for Agriculture, Forestry and National Resources Research and Development (PCARRD), which started a national program of rapid composting and the use of compost as fertilizer (*Zarate and Cardenas 2007*). Other species of *Trichoderma* can also serve as a biofertilizer, through the enhanced yield obtained with its use (*Cuevas 2006; Arpana and Bagyaraj 2007 and Singh 2010*).

The use of biofertilizers in crop production has greatly

increased in the last decade with the heightened consciousness to shift to ecological approaches in farming and the increasing awareness to take care of our environment. The current flagship agenda of the Department of Agriculture (DA) of the Philippines that calls for sustainable agriculture practices, which include among others, the reduction in the use of chemical fertilizers and pesticides in crop production, boosted the popularity of biofertilizers. The documented increasing ill effects on health and the environment with continuous use of these chemical inputs has pushed for the approval and implementation of the Philippine Organic Agriculture Law.

Alternative systems are rising due mainly to the need to counter and mitigate the serious negative effects of using synthetic fertilizers on people and the environment. This "back-to-basics" approach to soil fertility management had also shifted to an approach to pest management, which is more environment-friendly. All these considerations integratively aim to help improve the soil, enhance the health and economic status of the farmers and the environment and most importantly, encourage or maintain biodiversity in the land.

Such popularity of biofertilizers, which includes *Trichoderma* microbial inoculant (TMI), needs close scrutiny. There is a need to investigate its effect on other components of the agroecosystem. Under temperate conditions, *Grosch et al.* (*2006*), addressed the effects of *Trichoderma* on microbial communities using DNA-dependent SSCP (Single Strand Conformation Polymorphism) analysis of 16S rDNA/ ITS sequences. Recently, *Cordier and Alabouvette* (*2009*) studied the impact of the introduction of a biocontrol strain of *Trichoderma atroviride* on non-target soil microorganisms using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analyses of 16S and 18S rRNA genes. However, there are no local studies on the impact of *Trichoderma* applied on vegetable crops on the indigenous soil microflora.

Trichoderma microbial inoculant (TMI) application in Benguet revealed that there was a significant increase in yield in *Trichoderma*-treated cabbage with the incidence of some of the diseases of cabbage and other crucifers reduced or suppressed. Most importantly there was also a significant decrease in the infestation of insect pests. This resistance/ tolerance to insect pests was not documented, thus, this study was conducted to gather evidence on this added positive effect. The most significant impact, therefore, of this technology is not only as biofertilizer, biofungicide, growth promoter, and activator for rapid composting agent (*Cuevas 2006*; *Cuevas 2009*; *Cuevas and Bul-long 2009*; *Cuevas et al. 1995*, *Cuevas et al. 2001*; *Cuevas et al. 2005*) but also as possible biological control agent against insect pests.

In the past, only few or very limited data on this kind of alternative management options have been done under conditions and it was only in recent years that these alternative options were tried against insect pests (*Caasi-Lit 2010*). Likewise, observations were reported with the increase in yield and beneficial organisms when crops were grown in pesticide-free environment (*Caasi-Lit 2006*; *Caasi-Lit et al. 2008*) and treated with biofertilizers (*Altieri 1994*). The resistance and/or tolerance of these crops had apparently enhanced and encouraged the presence of the beneficial natural enemies like predators and parasites, thus, encouraging biodiversity.

Among the crucifers, cabbage is the most widely grown crop in mid- and high elevations in the Philippines. According to the *Bureau of Agricultural Statistics (BAS)* (*2009*), cabbage is planted in more than 7662 ha with a volume of production estimated at 124,712 Mt. In monetary terms, cabbage contributes about PhP 586.18 M to the country's annual revenue. It is a versatile vegetable and has very high fiber and mineral contents and is a rich source of vitamin A, calcium and antioxidants. Cabbage is also one of the main ingredients of the popular local Chinese dish called "chopsuey", a favorite vegetable dish of many Filipinos. In the Philippines, it is always a notion that when one eats "chopsuey", the ingredients came from vegetables that have been heavily sprayed with pesticides. This is attributed to the fact that pests and diseases remain the number one constraint in cabbage production and the most effective and reliable control measure against these pests for many farmers is the use of synthetic pesticides. In most growing areas, crucifers or upland vegetables are heavily sprayed with pesticides and this crop group has always registered the highest insecticide-usage in the country.

With the growing nationwide use of *Trichoderma* in crop production, it is important to know the impact of *Trichoderma* spp. introduction on the overall soil microbial diversity and its correlation with crop yield/performance and resistance to insect pests and diseases.

In encouraging farmers to shift from conventional to more eco-friendly practices like the use of *Trichoderma*, experiments conducted in farmers' fields offer the advantages for both scientists and farmers. *Cuevas et al.*, (*2011*, *2012*) have demonstrated under field conditions using a farmer- managed research approach that use of TMI in the control of club root disease of crucifers in Benguet increased profitability of cabbage cropping from increased yield and reduced material and labor inputs. In this type of research approach the researchers acquired insights on the risks vegetable farmers face from crop failure due to pests and diseases. Hence, the present study using the same farmer-managed research was conducted on a different location and focused on other components of the agroecosystem i.e. insect and microbial population dynamics as affected by the use of TMI. Monitoring the populations of these groups

of organisms are equally important factors in pest and disease management. With this research approach scientists gain hands-on experience in performing and setting-up experiments in actual farmer's field and conditions, with the farmer-collaborator still managing and deciding over activities in his farm. Such conditions, whether physical, biological and socio-economic, are environmental realities that both scientists and farmers have to contend with toward sustainability. On the part of farmers, particularly those with limited capabilities for external inputs, observing and evaluating results of experimental treatments first-hand and hand-in-hand with the researchers, is a learning and empowering experience. The disadvantages obviously are the lack of control over some variables like limitations on the sizes of plots, nature of borders and interplot spaces, something that is available in conventional researcher-managed experiments. The latter can be balanced by evaluating results using stricter criteria and employing appropriate statistical analyses.

This study aimed to evaluate the effects of the *Trichoderma* biofertilizer on cabbage in a farmer's field, particularly on the occurrence of or its reaction to major insect pests as well as determine the arthropod profile of cabbage under pesticide-free/*Trichoderma*-treated and pesticide-sprayed conditions. Also, determine the effect on the soil microbial dynamics as affected by the introduction of *Trichoderma* species using culture-dependent methods.

MATERIALS AND METHODS

Experimental site and treatments made

The study was conducted in a farmer's field on the slope (1000 m elevation) of Mt. Banahaw, Brgy. Sampaloc 1, Sariaya, Quezon. The field is surrounded by adjacent farms planted with peanut (north), upland rice (south), cabbage (west) and sweet potato (east). Cabbage (*Brassica oleracea*, *capitata* group var. 'Lucky Ball') seeds were sown in a seedbed and seedlings were transplanted in rows in two main treatments (*Trichoderma*-treated, T1 and Control, Farmer's Practice (FP), insecticide-sprayed plots, T2). The plots were laid out following randomized block design with each treatment replicated three times (**Figure 1**). The seedlings were planted at a distance of 0.3 m between rows and between hills. Each plot had 15 rows during the first trial and 21 rows during the second trial. There were at least six buffer rows between the two treatments. All the necessary cultural management practices in growing cabbage were employed except that no insecticide was applied in *Trichoderma*-treated plots. The study was conducted from June 1, 2010 to December 31, 2010 for two consecutive cropping seasons.

The TMI in powdered form developed by Dr. V. C. Cuevas, consisted of two strains of *Trichoderma ghanense*

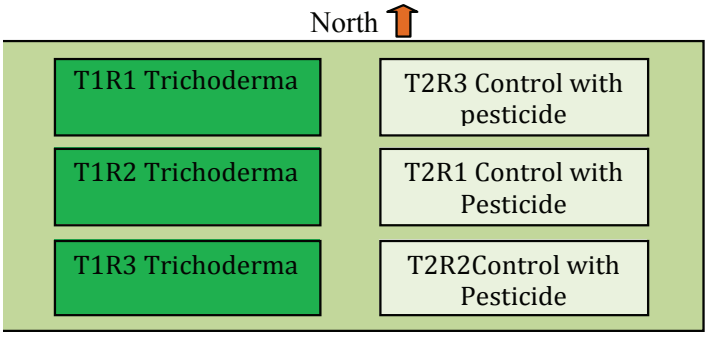


Figure 1. Randomized complete block design for each treatment in the experimental field in Sampaloc 1, Sariaya, Quezon. (T= Treatment; R= Replication).

Doi (formerly identified as *T. pseudokoningii* Rifai) and one strain of UV-irradiated *T. harzianum* Rifai mixed in equal proportions. These species of *Trichoderma* are naturally-occurring, free-living fungi that are components of soil and litter-root environments. The product is commercially produced by BIOSPARK Corp., UPLB Science Park, College, Laguna, a Filipino company that signed a licensing agreement with the University of the Philippines Los Baños. It is sold in 250-g packs; 1 g contains 1.6 x 10^8 colony-forming units (CFU) of *Trichoderma* spp.

During the first season, TMI was applied twice in T1- mixed with chicken manure in seed bed three days prior to seed sowing and seed coated prior to sowing. The rate of inoculant application was 0.5 g m^{-2}. During the second season, the inoculant was similarly applied but was repeatedly sprayed onto cabbage seedlings for 3 weeks to substitute for chemical sprays on the control plots due to heavy cabbage webworm infestation. Seed bed of T2– control was also applied with chicken manure but with no inoculant. Chicken manure and chemical fertilizer were both applied in the two treatments with the TMI treated receiving much reduced amount - only about 17% of chicken dung and 30% of chemical fertilizer in the first season (**Table 1**). During the second season, the reduction was only 20% for chemical fertilizer and 40% for chicken manure. The farmer-cooperator made this decision due to the very heavy infestation of the webworm.

Sampling and data analyses

Above-ground terrestrial arthropods were collected at 40, 60 and 85 days after transplanting (DAT) using ocular inspection (or visual counting), sweep net, pitfall traps and sugar and tuna baiting techniques. Soil samples for monitoring total culturable microbial population dynamics and community microflora structure were collected concurrently with arthropod sampling except that samples for microflora were also collected at 0 DAT but not for arthropods. Leaf damage of the test plants was assessed at different sampling periods (40, 60 and 85 DAT). The accumulated damage by

Table 1 . Approximate area and material inputs for the TMI and FP plots

Items	Season 1		Season 2	
	With TMI	FP (Chemical pesticide applied)	With TMI	FP (Chemical pesticide applied)
Approximate area (m^2)	100	100	100	100
Chem. fert- 14-14-14 applied (kg)	8	25	11	14
Insecticide applied (mL)	NA	Selecron 500 mL	NA	Selecron, Prevathon
Quantity of seedlings (g)	20	30	25	25
Quantity of insecticide (mL)	NA	500 Selecron	NA	125 Selecron; 125 Prevathon
Quantity of chicken dung (kg)	35	200	60	100

the dominant pest species was assessed. Percent leaf damage was computed by dividing the number of damaged leaves per plant over the total number of leaves per plant times 100.

All the arthropod specimens were sorted, identified and classified to functional guilds namely predators, parasites/ parasitoids, neutrals, sucking herbivores and chewing herbivores and the number of individuals composing each guild was counted and tabulated. Unidentified specimens were kept in separate Eppendorf™ tubes and labeled accordingly for future identification. The frequency of occurrence of the arthropods in the two treatments was computed, tabulated according to insect orders and functional guilds vis-a-vis the methods of insect collection. The results were used to compute for Shannon index. Only the data for sweep net and ocular methods were subjected to diversity index analysis (*Odum and Barrett 2005*). Data for the two seasons were analyzed using repeated measurements analysis of variance (RM ANOVA) using SAS PROC GLM (*SAS Institute 1997*). Statistical differences between treatments were tested using the F value and significant treatment differences using the Tukey's test.

Soil dilution spread-plate technique was used for monitoring microflora populations. Ten-gram soil samples from rhizosphere of four cabbage plants were used in 10-fold serial dilutions of the supernatants in sterile purified water. Aliquots were spread-plated in triplicate in nutrient medium amended with cyclohexamide 0.1 mg ml^{-1} for bacterial counting and spread-plating in Potato Dextrose Medium supplemented with 10% tartaric acid for fungal count. Colonies were counted after suitable incubation period (48 hours for bacteria and 5 days after inoculation for fungi) at ambient root temperature (28°C). BIOLOG Ecoplates with 31 Carbon sources were used for observation of microflora community structure. Methods for the extraction of microbial cells from the samples were according to the protocol of *Calbrix et al.* (*2004*) with a few modifications. Ten grams of rhizosphere soil were mixed with 90 ml of sterile purified water in a plastic conical tube. The mixture was vigorously shaken manually for 5 minutes, centrifuged at 129 x g (~1300 rpm) for 3 minutes and diluted to 10^{-2} with sterile purified water in a final volume of 20 ml for each microplate corresponding to approximately 10^5 cultivable microorganisms based on previous plate counts. This amount delivered around 1,500 CFU/well on the Ecoplate. The plates were then incubated at 28°C for 3 days. The OD590 was read after 17, 33, 40 and 60 hours using the MicroLog BIOLOG microplate reader.

Data collected during first season cropping included percent disease incidence, above-ground biomass production (kg), marketable yield (kg), and soil nutrient content analysis before and after the season were done. Cabbage plant tissues at 85 DAT were analyzed for total nitrogen and phosphorus content and corresponding nutrient uptake were computed.

Statistical analysis for culturable soil microflora population dynamics, biomass and marketable yield was done using Minitab, where Analysis of Variance was done using General Linear Model. BIOLOG results were analyzed by multiple regression analysis. The average well color development (AWCD) of each Ecoplate at each particular time was read and data were used for computation of the Shannon indices of diversity and evenness. Krustal-Wallis was used for non-parametric test for treatment mean differences. Principal component analysis was also computed using Eigen analysis of the correlation matrix.

RESULTS AND DISCUSSION

Arthropod profile and population dynamics

The arthropod compositions by insect order for two seasons in *Trichoderma*-treated and control (chemically-protected) cabbage plots in the farmer's field are presented in **Table 2** and **Figure 2**. The total number of arthropods collected from the five sampling methods (sweep net, ocular or visual counts, pitfall trap, sugar and tuna baits) was numerically higher in the control plots during the first trial (6,810) compared to the *Trichoderma*-treated plots (5,772). Meanwhile, during the second trial the *Trichoderma*-treated plots is higher (7,598) compared to the control plots (5,489). The overall total number of arthropods in *Trichoderma*-treated plots (13,370) was numerically higher when results from both trials were combined. More arthropods were collected during the second trial (13,087) compared to the first trial (12,582) (**Table 2**). Arthropod diversity and species richness was not significantly different in both treatments.

Table 2. Summary of the different parameters in the terrestrial arthropods profile on two season cabbage- cropping under *Trichoderma*-treated and chemically protected conditions.

Parameters	T1 with TMI	T2 – FP – chemical control
Terrestrial arthropod counts		
Total number of individuals		
First trial	5772	6810
Second trial	7598	5489
Both trials	13370	12582
Diversity Index		
First trial	1.187	1.117
Second trial	1.283	1.375
Both trials	1.235	1.246
Species richness		
First trial	1.435	1.409
Second trial	1.596	1.503
Both trials	1.515	1.456
Leaf damage, %		
First trial	49.27	43.77
Second trial	41.72	51.33

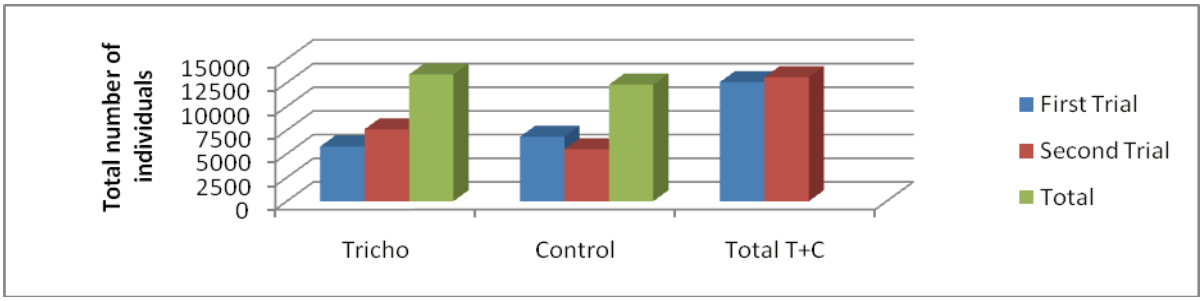


Figure 2. Total number of individual arthropods collected in *Trichoderma*-treated and control cabbage plots from the two trials using five sampling methods (sweep net, ocular, pitfall trap, sugar bait and tuna bait) in Sampaloc 1, Sariaya, Quezon. (Tricho/T = With *Trichoderma*; C = Control).

The *Trichoderma*-treated plots had significantly higher number of predators during the third sampling (85 DAT) during the first season, and of parasites during the first sampling (40 DAT) during the second season (**Tables 3 A** and **B**). The number of sucking and chewing pests was higher in the FP plots, despite the fact that chemical insecticide was applied. Predators were mostly beetles, spiders and ants. Parasites were mostly wasps (Hymenoptera). Assorted neutrals, either scavengers, vagrants or transients, were present in both plots.

Comparing leaf damage for the two treatments during the first season, the *Trichoderma*-treated plots were significantly higher during the pre-reproductive stage (**Table 4**). However, there was no significant difference in leaf damage during the reproductive stage in both treatments. The higher percent damage observed in *Trichoderma*-treated plots could be due to greater number of pests counted (**Table 2**). Even spraying of pesticides in FP does not prevent the reinfestation of the insect pests. This situation implies that chemical or pesticide spraying will likely increase pest population due to the suppression of natural enemies while *Trichoderma*-treated plots probably encourage build-up of natural enemies due to the absence of pesticide spraying. The vegetation surrounding the experimental plots may have contributed to the level of beneficial arthropods as they become refuges for natural enemies. The most predominant weed species in the surrounding vegetation were *Ipomoea batatas*, *Eleusine indica* and *Synedrella nodiflora* (data not shown). As mentioned above, *Trichoderma*-treated plots during the first season had higher damage at the pre-reproductive stage, but this treatment still produced marketable yield higher than the control (**Table 5**). It is possible that cabbage plants in the *Trichoderma*-treated plots were able to tolerate herbivory better than those in the control (pesticide-treated) plants.

The leaf damage during the second season was different. There was serious damage caused by the cabbage webworm on the TMI plots and damage was significantly higher than in the FP plots (**Table 4**). A one-week old whorl completely eaten and the larvae continued to bore deep into the inner tissues producing a hole in the core (**Figure 3 B** and **Figure 3 A**). With weekly spraying of TMI the cabbage plants that were damaged during the early vegetative stages were able to subsequently compensate whatever damage was incurred with their luxuriant growth and development (**Figures 3 C** and **D**). Compensatory growth has been well documented

Table 3 A. Mean number of insects by guild, treatments and sampling dates in cabbage during the first trial Sampaloc 1, Sariaya, Quezon (June-September 2010) Log transformed data [log (x+1)] were used in the RM ANOVA.

Guild 1	Treatments	1-FR		2-SR		3-TR	
Pest-chewing	w tricho no pesticide	7.33	a	19.13	a	17.27	a
	w/o tricho w/ pesticide	9.00	a	17.80	a	32.00	a
	Methods						
	1-SweepNet	2.17	b	6.83	b	19.00	b
	2-Ocular	38.33	a	85.50	a	104.17	a
	3-Pitfall	0.33	c	0.00	c	0.00	c
Guild 2							
Pest-sucking	Treatments						
	w tricho no pesticide	38.93	a	57.80	a	63.60	a
	w/o tricho w/ pesticide	23.63	a	46.75	a	94.81	a
	Methods						
	1-SweepNet	1.17	b	0.83	a	6.17	a
	2-Ocular	159.17	a	268.33	b	405.50	b
	3-Pitfall	0.00	c	0.00	c	0.17	a
Guild 3							
Predator	Treatments						
	w tricho no pesticide	3.00	a	58.20	a	34.60	a
	w/o tricho w/ pesticide	2.44	a	16.06	a	31.06	b
	Methods						
	1-SweepNet	9.17	a	6.50	a	13.33	a
	2-Ocular	4.83	a	13.33	a	19.00	a
	3-Pitfall	0.00	b	8.33	a	15.50	a
	4-Sugar	0.00	b	18.50	a	2.50	b
	5-Tuna bait	0.00	b	121.43	a	102.00	a
Guild 4							
Parasite	Treatments	**1-FR**		**2-SR**		**3-TR**	
	w tricho no pesticide	1.13	a	3.06	a	2.69	a
	w/o tricho w/ pesticide	0.87	a	1.67	a	3.00	a
	Methods						
	1-SweepNet	5.17	a	9.83	a	13.00	a
	2-Ocular	0.00	b	1.00	b	1.33	b
	3-Pitfall	0.00	b	1.29	b	0.29	b
Guild 6							
Neutral	Treatments						
	w tricho no pesticide	11.73	a	10.67	a	28.47	a
	w/o tricho w/ pesticide	7.93	a	9.47	a	28.40	a
	Methods						
	1-SweepNet	48.67	a	40.83	a	133.33	a
	2-Ocular	0.50	b	1.83	b	2.50	b
	3-Pitfall	0.00	c	7.67	b	6.33	b

FR = First Reading; SR = Second Reading; TR = Third Reading

as a response to herbivory (*Trumble et al. 1993*; *Thomson 2003; Molles 2010*). It is also probable that below- and above-ground herbivores had also an effect on the growth and development of the cabbage plant as reported by *Poveda et al. 2003*). The weekly spraying of *Trichoderma* may have promoted the growth of the plants, thereby regenerating the damaged core or whorl and producing cabbagelets or smaller heads. The cabbage plant was able to regenerate and produce another head, until it recovered up to the pre-harvest period (**Figures 3 E-F**). The plants in the control plots were not able to recover anymore and did not produce any head (**Figure 4**).

Nutrient content and uptake

The N content of the cabbage tissues was not significantly different between treatments but N uptake was significantly higher in TMI-treated plants (**Table 6**). P tissue content and uptake were not also significantly different between the control and TMI crops. However, in all parameters mentioned, slightly higher values were obtained in plants with *Trichoderma*. It should be noted that the amount of fertilizers applied in TMI plots were much reduced compared to FP. *Harman et al.* (*2004 and 2008*) reported that one of the many benefits of using *Trichoderma* is the reduction of use of N fertilizer in corn without yield

Table 3B. Mean number of insects by guild, treatments and sampling dates during the second trial, Sampaloc 1, Sariaya, Quezon (September-December 2010). Log transformed data [log (x+1)] were used in the RM ANOVA.

Guild 1	Treatments	1-FR		2-SR		3-TR	
Pest-chewing	w tricho no pesticide	91.166	a	57.833	a	44.166	a
	w/o tricho w/ pesticide	21.166	a	34.000	a	46.666	a
	Methods						
	1-SweepNet	3.667	b	4.833	b	2.000	b
	2-Ocular	108.667	a	87.000	a	88.833	a
Guild 2	Treatments						
Pest-sucking	w tricho no pesticide	424.167	a	330.000	a	0.666	a
	w/o tricho w/ pesticide	92.667	b	70.167	b	5.000	a
	Methods						
	1-SweepNet	2.667	b	0.167	b	0.5000	b
	2-Ocular	514.167	a	400.000	a	5.166	a
Guild 3	Treatments						
Predator	w tricho no pesticide	69.133	a	9.2667	a	26.6	a
	w/o tricho w/ pesticide	207.533	a	10.9333	a	16.8	a
	Methods						
	1-SweepNet	0.667	c	9.333	b	5.500	b
	2-Ocular	27.833	b	41.166	a	103.000	a
	3-Pitfall	18.167	b	0	c	0	c
	4-Sugar bait	12.667	bc	0	c	0	c
	5-Tuna bait	632.333	a	0	c	0	c
Guild 4	Treatments						
Parasite	w tricho no pesticide	7.000	a	1.777	a	0.111	a
	w/o tricho w/ pesticide	3.111	b	1.000	a	0.111	a
	Methods	1-FR		2-SR		3-TR	
	1-SweepNet	0.166	a	0.833	b	0.333	a
	2-Ocular	14.666	b	3.333	a	0	a
	3-Pitfall	0.333	a	0	b	0	a
Guild 6	Treatments						
Neutrals	w tricho no pesticide	5.444	a	18.666	a	3.222	a
	w/o tricho w/ pesticide	6.000	a	15.333	a	6.666	a
	Methods						
	1-SweepNet	8.833	a	2.333	b	4.833	a
	2-Ocular	0.333	b	48.666	a	10.000	a
	3-Pitfall	8.000	a	0	b	0	b

FR = First Reading; SR = Second Reading; TR = Third Reading

reduction, and they attributed such effect to increased root growth and nutrient uptake and fertilizer utilization efficiency. In this particular study, the increased nutrient uptake was translated into higher biomass and marketable yield of the TMI-treated crops.

Biomass and yield of cabbage

Monitoring of weights of above-ground crop biomass was done at 40, 60 and 85 DAT during the first season. *Trichoderma*-treated plants had slightly greater biomass gain than the control plants at all growth stages (**Figure 5**). At harvest, total and marketable yield components were higher in the TMI plots. There was 26% increase in marketable yield with the application of *Trichoderma* with reduced dose of fertilizers compared to the control plants. However, yield differences were not significant during the first season.

Soil chemical analysis

Soil chemical analyses at the beginning and end of the first season showed that the soil in the TMI plots had higher levels of organic matter (OM) and nitrogen (N) based on treatment means. The control plots had higher pH and phosphorus (P) content. The two treatments had relatively the same levels of potassium (K). Looking at general trends, soil pH and OM significantly increased from the start to the end of the experiment (**Figure 6**). There were no significant differences between the initial and final levels for N, P and K.

Disease Monitoring

The dominant symptom for signs of disease cabbage plants was wilting of the leaves, especially during the early stages of growth, i.e., at 40 DAT but the incidence decreased with time until no plants were observed wilting at 85 DAT

Table 4. Leaf feeding damage of cabbage in Trichoderma-treated and control plots at different sampling periods during the first and second trial in Sariaya, Quezon.

Treatment	40 DAT vegetative stage	60 DAT pre-reproductive stage	85 DAT reproductive/ harvest stage
Season 1			
1 *Trichoderma*	50.333 a	44.666 a	52.833 a
2 Control	46.333 a	31.166 b	53.833 a
Season 2			
1 Control	41. 830 b	36.330 a	75.830 a
2 *Trichoderma*	54.830 a	39.500 a	30. 830 b

Means in the same column followed by a common letter are not significantly different at 10% for the treatment and at 5% for the trial

Table 5. Combined analysis of yield of cabbage in TMI-treated and control plots during the first and second trial in Sampaloc 1, Sariaya, Quezon.

Treatment	Yield (kg)	SE Mean
1 Control	4019.17 b	1149.08
2 *Trichoderma*	5391.33 a	1279.07

Means in the same column followed by a common letter are not significantly different at 5%

Table 6. Nutrient content and uptake of cabbage plants at harvest (85 DAT) as affected by TMI application.

Treatments	N-content (%)	N-uptake (mg g^{-1})	P-content (%)	P-uptake (mg g^{-1})
Control	0.80 a	11.00 b	3,030.70 a	43,157.00 a
Trichoderma	0.99 a	18.33 a	3,297.00 a	60,506.00 a
Nutrient means	0.89	14.67	3163.85	51831.50

(data not shown). Based on the baiting experiments, the causal organisms belonged to genus *Pythium*.

Total bacterial plate counts

Total microbial counts determined by spread-plating of rhizosphere soil samples in culture media taken from day 0 (initial), 40, 60 and 85 DAT showed trends (**Figure 7**). As expected, bacterial count (10^9) was several folds higher than the fungal population at only 10^5. Bacterial counts of the control plants from time 0 increased from 1 x 10^8 to 1.6 x 10^9 at 60 DAT and then to 4.4 x 10^9 at 85 DAT. The *Trichoderma*-treated plots also had increased in bacterial population up to 1.6 x 10^9 at 85 DAT. There were no significant differences in the bacterial counts of the control and *Trichoderma*-treated plots, except in the control at 85 DAT (**Figure 7**).

Fungal plate count

Meanwhile, fungal plate count varied from an initial count of 10^5 at transplanting time then decreased to 10^4 at 85 DAT for the control and *Trichoderma*-treated plots (**Figure 7**). The increase in fungal population in the *Trichoderma*-

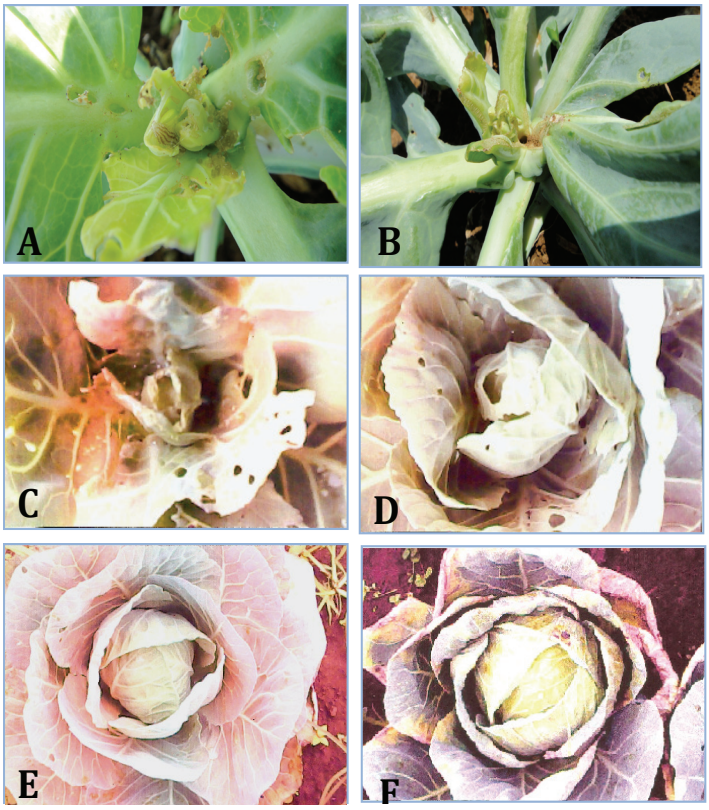


Figure 3. Early vegetative damage by the cabbage webworm, *Hellulaundalis* on the whorl (A, B) and how the cabbage plant recovered from the damage (C-F).

Figure 4. Cabbage crop damaged by webworm from FP plots sprayed by Selecron and but was not able to recover.

treated plots from 40 to 60 DAT is expected due to the application of fungal spores, succeeding germination and increase in number of colonies. *Trichoderma* colonies were also observed in plates of the control plots, which showed that potential indigenous *Trichoderma* species are also present. The decreased in fungal population at 85 DAT is possibly due to the lesser nutrients in the rhizosphere soil due to the uptake of the plants and older matured roots and less slough off tissues, hence lesser nutrients for the microbes.

Plating macerated cabbage roots from *Trichoderma*-

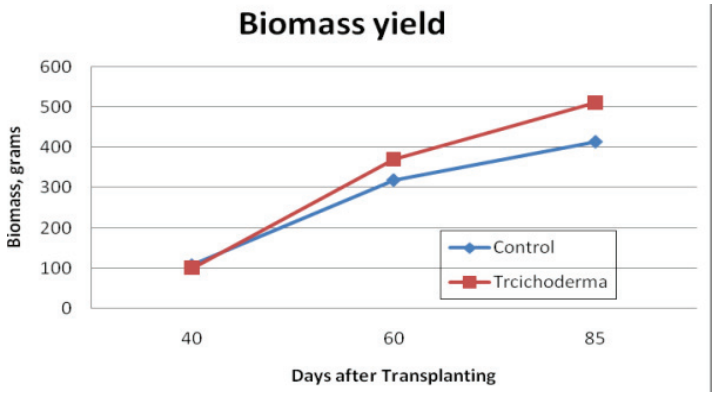
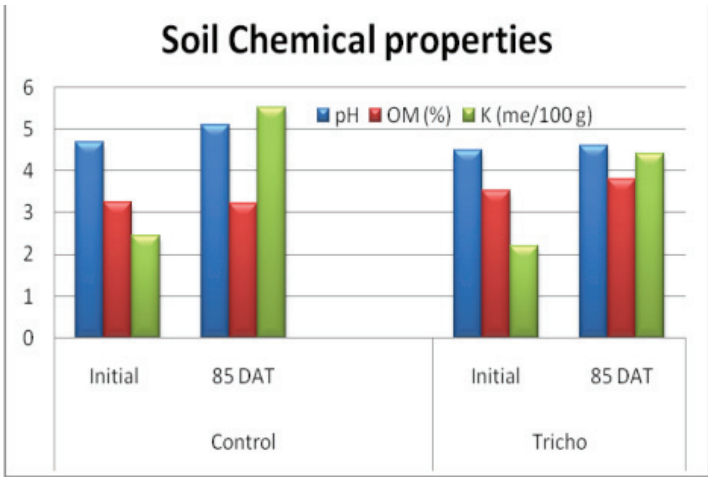


Figure 5. Biomass of cabbage plants in response to *Trichoderma* application during the growth stages of the crop.



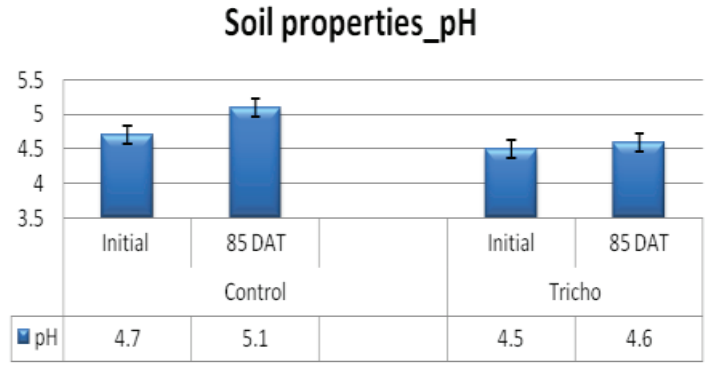
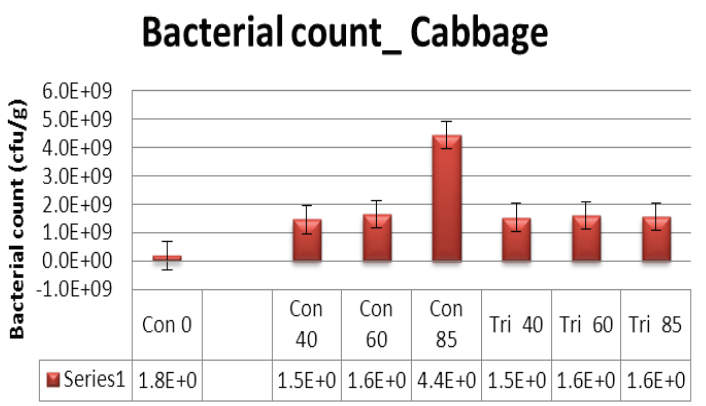


Figure 6. Graphical representation of the soil chemical properties as affected by *Trichoderma* application in the beginning and at the end of the study.

treated plots showed the presence of *Trichoderma*-like colonies corroborating earlier studies that *Trichoderma* can also form endophytic associations.

Microbial community structure as analyzed by BIOLOG Ecoplates

Among the several techniques currently available to analyze soil microbial communities, the BIOLOG (Biolog, Hayward, CA, USA) approach, based on utilizable patterns of a wide range of single carbon sources (*Garland and Mills*



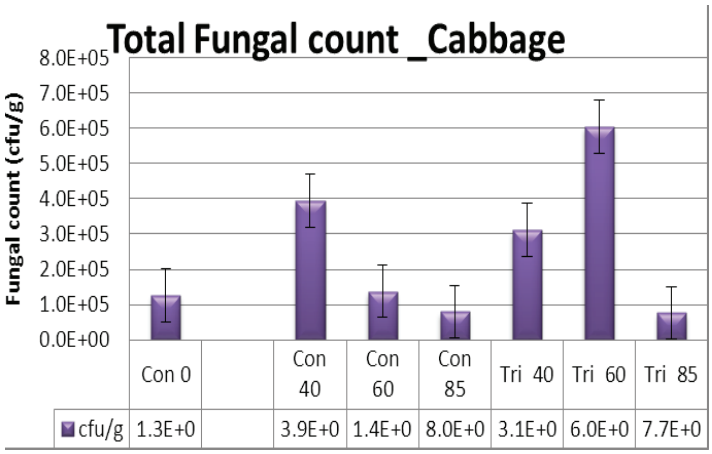


Figure 7 . Total bacterial and fungal plate count in the control and *Trichoderma* applied plots as monitored through time.

1991) provides community level physiological profiles (CLPPs) by analysis of the number of substrate types utilized (diversity of metabolic potential) or response to individual substrates or substrate guilds. Changes in microbial community as affected by *Trichoderma* application versus chemical pesticide application was measured by a color development assay performed on BIOLOG microtitre plates. Metabolic reactions were measured by the change of the dye from colorless to purple (individual well color development), indicating bacterial and fungal heterotrophic growth on the specific substrate as a carbon and energy source. Optical density readings obtained for average well color development (AWCD) and total well color development (TWCD) showed an increasing value from 17 hrs to 60 hrs of plate incubation suggesting microbial community growth and utilization of the substrate (**Table 7**).

When the individual carbon sources were compared according to treatment, there was no particular difference in the Shannon diversity and evenness indices between the control plots and the *Trichoderma*-treated plots. The similarity in the diversity and evenness indices was observed all throughout the growth stages of the crop from 40 to 60 and up to 85 DAT.

In terms of diversity index as affected by time of plate incubation, the shorter the time of incubation (17 hrs), the lower is the diversity index and this increases with longer

Table 7. Multiple regression analysis of all variables in BIOLOG Ecoplates.

Predictor	Average Well Color Development (AWCD)	Total Well Color Development (TWCD)	Number of Wells with color Development (S)	Shannon Diversity Index (H)	Shannon Evenness Index (E)
Constant	-0.338 **	-12.71**	7.22**	2.022**	0.90**
Treatments a	-0.065 n.s.	-1.74 n.s.	0.18 n.s.	-0.001 n.s.	0.003 n.s.
Days	0.002**	0.075**	0.04**	0.0014n.s.	-0.0005 *
Incubation	0.037**	1.136**	0.368**	0.024**	0.002**
R-Sq(adj)	90.7%	90.7%	78.3%	65.1%	28.9%

a Treatment dummy variable: 0 = control; 1= *Trichoderma* applied

time of incubation, the highest at 60 hrs of incubation. However, Shannon evenness index was similar between seperate treatments and was not affected by time of plate incubation.

Correlation analysis of Biolog data

Incubation period, AWCD, TWCD S, H and E were significantly correlated with each other, where S is the number of plates showing color development (**Table 7**). Treatments had no significant correlation with the other variables as shown in the multiple regression analysis. *Trichoderma* application was similar to the control, in that there was no effect in the diversity of microbes (**Table 7**). For the two treatments and in any given day levels of AWCD, TWCD, S, H and E increased with longer incubation period. These parameters increased from 40 to 60 DAT then decreased toward 85 DAT in the control treatment. The daily trend in the *Trichoderma*-treated plots did not change much. The Shannon diversity (H) and evenness (E) indices dropped sharply from 50 to 85 days in both the *Trichoderma*-treated and control. There was no significant difference between treatments (control vs *Trichoderma*) as indicated by the non-significant partial regression coefficient of treatments, and the Kruskal-Wallis non-parametric test for treatment differences. These community microflora data, therefore, indicated that bacterial and fungal communities were not significantly affected by *Trichoderma* application.

Bacteria and fungi play critical roles in the formation of soil aggregates in good soil structure and the biological control of root diseases. Both promote the detoxification of soil contaminants and the production of plant growth promoters and organic chelating agents. Population densities assessed through soil–plate dilution, showed that *Trichoderma* inoculation caused a slight increase in the fungal population from an initial 1.3 x 10^5 cfu g^{-1} to 6 x 10^5 cfu g^{-1} at 60 DAT, while fungal population was highest in the control at 3.9 x 10^5 cfu g^{-1} at 40 DAT and decreased with time. There was a clear fungal population growth immediately after the addition of *Trichoderma* with its effect lasting from day 0 until 60 DAT. At 85 DAT, no difference in fungal population was observed between control and *Trichoderma*-treated plots. Only transient shifts were observed after TMI introduction but there was no permanent impact on the indigenous fungal community. This result was also observed by *Cordier and Alabouvette* (*2009*) as determined by Terminal Restriction Fragment Length Polymorphism method based on 18S and 16S rRNA genes, whereby the introduction of *Trichoderma* strain I-1237 into

Table 8. Non-parametric test for treatment difference following Krukal-Wallis test using different variables in BIOLOG Ecoplates.

AWCD:

Treatment	N	Median	Ave. Rank
Control	44	1.95	40.1
Trichoderma	33	1.10	37.5
Overall	77		39.0

H= 0.27 n.s.; d.f. = 1 P= 0.607 (adjusted for ties)

TWCD:

Treatment	N	Median	Ave. Rank
Control	44	36.30	39.9
Trichoderma	33	33.29	37.8
Overall	77		39.0

H= 0.17 n.s.; d.f. = 1 P= 0.681 (adjusted for ties)

S:

Treatment	N	Median	Ave. Rank
Control	45	25.00	38.3
Trichoderma	33	25.00	41.1
Overall	78		39.5

H= 0.30 n.s.; d.f. = 1 P= 0.587 (adjusted for ties)

H:

Treatment	N	Median	Ave. Rank
Control	44	3.08	38.8
Trichoderma	33	3.10	39.2
Overall	77		39.0

H= 0.01 n.s.; d.f. = 1 P= 0.938 (adjusted for ties)

E:

Treatment	N	Median	Ave. Rank
Control	44	0.97	38.7
Trichoderma	33	0.96	39.4
Overall	77		39.0

H= 0.02 n.s. ; d.f. = 1 P= 0.888 (adjusted for ties)

soils slightly modified the microbial diversity, only for a short period of time. Nine months post- inoculation resilience took place, resulting in similar structures of the fungal and bacterial communities in the inoculated and control soils. Similar results were also obtained by *Bankhead et al.* (*2004*) following root colonization of wild type and transgenic biocontrol strains. Similarly, *Glandorf et al.* (*2001*) obtained similar results on genetically modified *Pseudomonas putida* on the fungal population and *Lottmann et al.* (*2009*) on the effect of *Rhizoctonia solani* introduction on the indigenous microbial community.

This result maybe attributed to the action of indigenous and introduced microbes (*Trichoderma*) to the abundant substrate in the form of organic matter (compost), which was applied to the crop at transplanting time. The enhanced secretion of enzymes (i.e. cellulases) hastened the degradation of the substrates and the secretion of growth hormones led to greater nutrition and faster growth of plants and ultimately to heavier yield.

The application of *Trichoderma* did not cause an increase or decrease in the culturable bacterial community through time. The population densities of the microbes are dependent on the initial substrates and released metabolites, which could influence the evolution of fungal and bacterial communities by selecting the microorganisms adapted to the new environment. *Trichoderma* species are known to secrete a large diversity of metabolites having antibacterial activities. Hence, proliferation of species resistant or tolerant to the metabolites released could have occurred. The identity of the indigenous population was not analyzed and is recommended to be done in future studies. However, markers or biological indicators should be available to fully monitor changes in microbial population.

There was no difference observed between the control and *Trichoderma* treatments in terms of utilization of the 31 carbon sources as indicated by the AWCD and TWCD. Should be Shannon and diversity and evenness indices suggested that *Trichoderma* application only affects the microbial population at the initial hours (17 hrs of incubation). However, with longer incubation, no more differences were observed. This result was similar to the work of *Cordier and Alabouvette* (*2009*) where the difference in the inoculated and control soils were significant only for 3 months after *Trichoderma* inoculation. At the end of the experiment, the fungal community structures of the inoculated and control soils were not different from each other.

The Importance of Integrated Studies on Terrestrial and Soil Environments

The studies on terrestrial arthropods and soil microbial population dynamics in cabbage as affected by the addition of TMI has significantly contributed to the basic knowledge on plant-*Trichoderma*-arthropod interaction. It is the first report on simultaneously studying these important components of the ecosystem. It is the first attempt to comprehensively explain directly or indirectly the effects of *Trichoderma* biofertilizer or microbial inoculant on herbivores and soil microbes in cabbage. Guided by the classical farming adage"Don't feed the plant, feed the soil", this integrated, ecologically-based pest and disease management approach as espoused by *Altieri and Nichols* (*2004*) is getting "back to basics" in crop farming. It is common knowledge that crops grown in organically rich and biologically active healthy soils are less susceptible to pests and diseases (*van Bruggen et al. 2006*).

Induced direct defense against insect herbivores is also triggered upon feeding by producing secondary metabolites. On the other hand, the induced indirect defence is achieved by producing a blend of volatiles that attract carnivorous enemies of the herbivores (tritrophic interaction). It would be important, therefore, to closely monitor and study chemical substances in the plant, which may change or fluctuate as a response to *Trichoderma* treatment. This might be helpful in explaining to farmers their future pest management options. *Harman* (*2011*) in his article "*Trichoderma*– not just for biocontrol anymore" emphasized the far-reaching ability of *Trichoderma* in strongly affecting the physiology of the plant by changing plant gene expression and improving plant performance and regulating disease and pest incidence.

CONCLUSIONS AND RECOMMENDATIONS

The study confirmed preliminary findings of previous studies on the numerous benefits of using TMI as biocontrol agent, plant growth promoter and inducer of increased nitrogen use efficiency. Nitrogen application can be reduced to 50% without reducing the yield of cabbage. It also demonstrated systemic resistance against the plant disease caused by Pythium. Microbial community structure was stabilized at the end of the cropping season. An encouraging result is the potential of *Trichoderma* to control insect pest populations in cabbage. Significantly, arthropod biodiversity is encouraged in the pesticide-free plots compared to the chemically treated plots. For cabbage, a succulent crop, *Trichoderma*-treated plants were able to tolerate herbivory better than the control. The plants were able to subsequently compensate whatever damage was incurred. The added benefits were clearly on the positive effects in regulating pest populations as well as encouraging the build-up of natural enemies in cabbage fields. The data generated in this study affirmed the previous observations in the *Trichoderma* trial sites in Benguet and Mountain Province. This document showed for the first time the positive pest management-related effect of *Trichoderma* in cabbage farming.

On the other hand, *Trichoderma* applied in the form of the commercially available inoculant Biospark™ significantly affected the growth and nitrogen content of plants treated with the inoculant, despite amendment of only half the chemical fertilizer as compared to the control that received a full dose. This was reflected in the greater marketable and total yield of cabbage from treated plots. Such biofertilizer effect of the inoculant can be due to the ability of *Trichoderma* to mineralize nutrients from soil organic matter. There were fewer wilted plants in inoculated plots compared to the control showing the biocontrol effect of the inoculant.

Soil dilution spread-plate technique done at 0, 40, 60 and 85 days after transplanting the crop showed only transient shifts after *Trichoderma* inoculation but there was no permanent impact on the indigenous fungal community. Similar trends where no difference was observed between the control and *Trichoderma* treatments in terms of utilization of the 31 carbon sources. Shannon diversity and evenness indices suggest that *Trichoderma* application only affects the microbial population at the initial hours (17 hrs of incubation). However, with longer incubation, no more difference was observed. At the end of the experiment, the fungal community structures of the inoculated and control soils were not different from each other. The application of *Trichoderma* did not cause an increase or decrease in the culturable bacterial community through time, while in the control an increase in bacterial population was observed toward the end of the crop growth. The use of markers or biological indicators is recommended to fully monitor changes in microbial population.

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