Effects of forest management practices on microbial biomass, litter decomposition, microbial abundance, and the soil's physical and chemical properties of replacement plantations after pine wilt disease

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ABSTRACT

The goal of this study was to determine the effects of two combinations of forest management practices employed on replacement pine plantations after pine wilt disease. The objective was to measure the soil's physical and chemical properties, microbial biomass, litter decomposition and microbial abundance as affected by thinning and pruning, and the understory weeding and applications of insecticide and nitrogen fertilizer. This study was conducted in Ryuosan, Higashihiroshima City, Japan. Results showed that the physical characteristics (color, texture, moisture and water-holding capacity) of the three study sites did not differ significantly from each other. On the other hand, the chemical properties (pH, C and N) showed significant differences among sites. The relative light intensity difference (LID) greatly fluctuated and differed. Microbial biomass and microbial abundance were shown to have seasonal variations and lower at the managed sites than at the unmanaged site whereas litter decomposition did not vary significantly. The high correlations among biological and physico-chemical properties of soil at the study sites implied high interdependence among soil's characteristics.

Keywords: forest management practices, microbial biomass, replacement plantations, microbial abundance, pine wilt disease.

INTRODUCTION

There were massive forestry losses, especially in Japan, due to pine wilt disease caused by pinewood nematode infestations (Mamiya 1983; Fukuda et al. 1998). With the occurrence of this disease, foresters and even private land owners have become more concerned about how to protect forest ecosystems from infestations. One of the solutions for protecting forests from infestations is the use of different forest management practices (Johnston and Crossley 2002). In similar process in Japan, the areas affected by pine wilt disease were

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totally deforested and replaced by plantations. These replacement plantations are then subjected to different management practices to prevent contamination and infestations and to increase commercial value of forest stands during their development (Rötzer et al. 2010).

Concerns have increased regarding the possibility of detrimental ecological effects of forest management practices (Johnston and Crossley 2002; Bird et al. 2004; Lundmark et al. 2017) and have been placed under scrutiny with respect to their impacts on the environment and on site productivity and biodiversity (Burger and Zedaker 1993; Gupta and Malik 1996; Günter et al. 2011). Many studies have been conducted to show the effects of forest management practices, like clear-cutting (Donegan et al. 2001; Gondard et al. 2003; Lundmark et al. 2017), forest harvesting (Marshall 2000; MacDonald and Thompson 2003; Bird et al. 2004), understory weeding (MacDonald and Thompson 2003), fertilizer applications (Lee and Jose 2003; Williamson and Neilsen 2003; Zhang et al. 2017), and prescribed burning (Pietikäinen and Fritze 1995; DeLuca and Zouhar 2000), on the soil's physical and chemical properties and biological components. Each of these forest ecosystems studied had their own history of disturbances, but there is no record yet of any study conducted on forest management practices after pine wilt disease.

Soil microorganisms have been proven to be sensitive indicators of environmental disturbances (Torsvik and Øvreås 2002). They are responsible for most soil processes but the knowledge of the relationship between them and aboveground processes is still incomplete (Colombo et al. 2016). Reports have been very varied about microbial responses to different disturbances. The disturbance history of the forest ecosystem may contribute to the varying responses of microorganisms towards different management practices (Johnston and Crossley 2002).

Two combinations of management practices were considered in this study, both employed in young pine plantations. The first combination was biomass thinning and pruning, and the second combination was understory weeding and applications of nitrogen fertilizer and insecticide. Biomass thinning and pruning which involve selective removal of small diameter trees and the cutting of the appropriate amount of branches, are practices traced with very long history (Forest management solutions 2004; Lundmark et al. 2017). Thinning and pruning are considered important management techniques for plantations, and are undoubtedly indispensable for the sustainable development of forest (Seiwa and Kikuzawa 1994; Ooishi et al. 1998; Krauchi et al. 2000; Montagu et al. 2003; Rötzer et al. 2010). Thinning and pruning creates canopy openness or gaps, which are critical in the community dynamics of many types of forest (Gray and Spies 1996). For example, tree harvesting and site preparation practices have resulted in a significant loss of nutrients and organic matter, thereby decreasing site

productivity (Pritchett and Fisher 1987; Bormann and Likens 1994). Another study conducted by Donegan et al. (2001) showed that clear-cutting decreased the populations of nematodes, microarthropods, bacteria and fungi in both litter and soil. In addition, clear cutting could have similar genetic effects as pest outbreaks, wildfires or storms (Alfaro et al. 2014).

Fertilization and understory weeding of forest plantations have also become an increasingly important part of intensive management in recent years (Allen et al. 1990; Fox 2000). The few existing studies have reported conflicting results of the effects of fertilization, some reporting positive effects (Gallardo and Schlesinger 1994; Williamson and Neilsen 2003), and others reporting negative effects (Haynes and Gower 1995; Lee and Jose 2003). These conflicting results could be attributed to differences in allocation patterns among tree species, soil condition, stand's age and disturbance history. Fertilization increased the arthropod abundance in the harvested site which was comparable to an unharvested site (Bird et al. 2004).

The goal of this study was to determine the effects of two combinations of forest management practices employed on replacement pine plantations after pine wilt disease. The objectives were to measure the soil's physical and chemical properties, microbial biomass, litter decomposition and microbial abundance as affected by thinning and pruning, and the understory weeding and applications of insecticide and nitrogen fertilizer.

METHODS

Study Site

This study was conducted in Ryuosan, Higashihiroshima City, Japan. Three study sites were chosen for this study. The first one (Figure 1) was a 6-year old stand (P6) maintained by under-storey weeding, application of nitrogen fertilizer and spraying of insecticide once a year, without pruning or thinning. The second site (Figure 2) was a 10-year old stand (P10) maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year. These two sites are pine plantations which are replacement stands after total deforestation and eradication because of pine wilt disease. The third site (Figure 3) was an unmanaged pine forest (UM) about 30 years old, which is not affected by the disease and without any previous history of pine wilt disease. In these study sites, three 10m x 10m plots were established. Sampling was conducted every other month for a period of 13 months.

Figure 1. A 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without thinning or pruning. During the first 4 years in this plantation, the under-storey grasses were removed but this was then stopped.

Figure 2. A 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year.

Figure 3. An unmanaged pine forest without any previous history of pine wilt disease.

Soil Sampling

For all the soil analyses conducted, top soil samples were taken from 12 different points, to a depth of 5 cm, randomly in each of the study sites using a sterile hand corer. Samples were then homogenized and big particles, such as litter, rocks and macrofauna, were hand-removed aseptically. Samples were then subjected to sieving (of desired mesh size depending on the analysis done). Sampling was done once every other month.

Light Intensity Difference (LID)

The relative light intensity in each study area was measured at noonday using an LI-210SA photometric sensor (LI-COR, USA). The light intensity, both outside and inside the forest, was measured, and the difference between the two was recorded as LID (light intensity difference).

Physical and Chemical Properties of the Soil

The moisture content was measured by oven-drying the samples at 105^{o}C for 24 hours. The color of the soil was determined through ocular inspection (Munsell 1976; Oyama and Takehara 1997). The water-holding capacity was measured using the Hilgard method (Childers et al. 1996). The soil texture was determined using the modified 'jam jar' method (Anonymous 2000; Gardeners Supply Company 2002). The pH at each site was determined using an electrode pH meter (Tateishi et al. 1989). The total carbon and total nitrogen were measured using a C-N analyzer (Tateishi et al. 1989). Except for

soil color and texture, all other physical and chemical analyses of soil samples were conducted bimonthly.

Litter Decomposition

Freshly abscised pine needles were collected in July 2003 from the study area. The litter samples were air-dried until constant weight was achieved. Five grams of litter was placed in nylon (1 mm mesh size) litter bags. These litter bags were exposed to UV light for 10 minutes in a laminar flow as a form of sterilization. The litter bags were placed randomly in each plot, where each bag was tied firmly to a stick and covered with litter/soil. Three replicates of litter bags from each plot were collected bimonthly to determine the mass loss. Mass losses were determined after oven-drying the samples to a constant weight at 80^{o}C.

Microbial Analyses

Microbial biomass carbon. The microbial biomass carbon was measured using the chloroform-fumigation and direct extraction method (Vance et al. 1987). This procedure was then followed by dichromate digestion and titration.

Microbial abundance. The dilution plate count technique was used in this study (Tateishi et al. 1989) to enumerate the functional groups of microorganisms using selective culture media. For Gram positive bacteria, an Albumin agar medium was used. The same Albumin agar medium was used for Gram negative bacteria but added with 10 ml.L^{-1} 5% crystal violet. Actinomycetes and fungi were enumerated using Dextrose-nitrate agar medium and Rose Bengal agar medium, respectively. For cellulase-producers and amylase-producers, the Most Probable Number (MPN) technique was used (Acea and Carballas 1996).

Three sub-samples were used from each plot and Petri plates were then incubated, at 28^{o}C for 2-5 days for bacteria and actinomycetes, at 25^{o}C for 7-10 days for fungi, and at 28^{o}C for 4-9 weeks for cellulolytics and amylolytics microbes. All the results were obtained in triplicates.

Data Analyses

All the data obtained were subjected to analysis of variance (ANOVA) to show the significant differences among means, and Tukey's test was employed to separate means. Correlation analysis was used to test the relationships between the litter decomposition, light intensity difference, microbial biomass and abundance, and the soil's physical and chemical characteristics.

RESULTS

Light Intensity Difference (LID)

The relative light intensity difference (LID) of P6, P10 and UM greatly fluctuated and differed from each other. The average LID at P6 was 3.75 klux, at P10 3.01 klux and at UM 4.36 klux (Table 1). The LID at UM was then around 19.5% and 30.9% greater than that at P6 and at P10, respectively.

Table 1. Mean light intensity difference (LID) and the physical and chemical properties of the soil.

Study sites	LID (Klux)	Color	Texture	Sand (%)	Silt (%)	Clay (%)	Moisture (%)	WHC (%)	pH	C (%)	N (%)
P6	3.75	10YR5/6	loam	52.2	31.1	16.7	26.2	66.77	5.97	2.14	0.207
P10	3.01	10YR4/6	loam	53.0	29.5	17.5	23.4	66.03	4.36	2.35	0.247
UM	4.36	10YR4/4	loam	51.3	29.5	19.2	27.5	68.3	4.9	4.85	0.302

Physical and Chemical Properties of the Soil

The soil color at P6 was yellowish brown (10YR5/6), at P10 brown (10YR4/6) and at UM brown (10YR4/4). The soil texture was loam for all three study sites (Table 1).

The soil moisture content at each site was high from April to July and low from December to February. The mean moisture content at P6 was 26.2%, at P10 23.4%, and at UM 27.5% (Table 1). The moisture content at UM was around 4.7% and 14.9% higher than that of P6 and P10, respectively.

The water holding capacity (WHC) in all study sites was almost constant throughout the whole year during which the WHC at the three sites did not differ significantly. The mean WHC at P6 was 67.77%, at P10 66.03% and at UM 68.3% (Table 1)

The pH was slightly acidic at all sites. The mean pH at P6 was slightly high, 5.97, probably because of fertilization. The mean pH at P10 was 4.36, while it was 4.9 at UM (Table 1). Tukey's test showed that the pH in P6 was significantly higher than that of P10 and UM.

The total carbon and nitrogen were higher in UM than in P6 and P10 (Table 1). At UM, the averages of soil carbon and nitrogen contents were 4.85% and 0.302%, respectively. At P6, the average soil carbon and nitrogen contents were 2.14% and 0.207%, respectively. For P10, the average soil carbon and nitrogen contents were 2.35% and 0.247%, respectively.

Litter Decomposition

The litter decomposition rate was faster at P10 but not significantly different from P6 and UM. At the end of 13 months, 40.3% of litter samples remained in P6, 36.7% in P10 and 45.8% in UM. The decomposition process underwent a fast initial phase, followed by a slow intermediate phase, and then a fast terminal phase (Figure 4). ANOVA showed no significant difference in decomposition rate between sites (Table 3). Litter decomposition also showed no correlation to any of the LID and the soil's physico-chemical properties (Table 2).

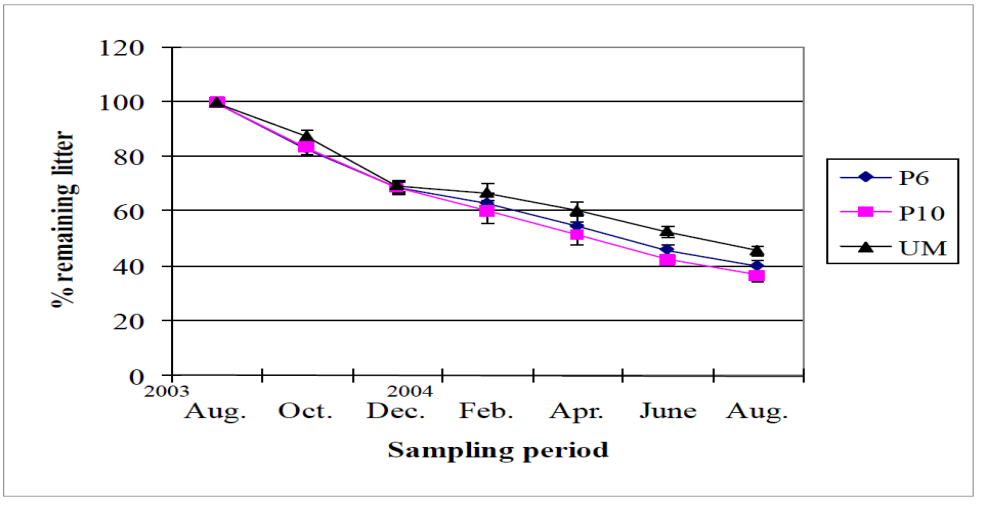

Figure 4. Average (±se) litter decomposition at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

Microbial Biomass Carbon

The ANOVA (Table 3) showed that the microbial biomass carbon differed significantly between the study sites ($p<0.05$). UM showed the highest microbial biomass carbon, followed by P10, and then P6. Tukey's test proved that P6 and P10 were not significantly different from each other, but they were significantly different from UM. High values were recorded in October 2003, and from April to June 2004. A little fluctuation occurred from December 2003 to February 2004, but the lowest value was recorded in August 2003 at P10 (Figure 5). Correlation analysis proved a significant relationship between biomass carbon and moisture content ($r^2=0.584$, $p<0.01$), LID ($r^2=-0.628$, $p<0.01$), total carbon ($r^2=0.568$, $p<0.01$), and total nitrogen ($r^2=0.760$, $p<0.01$).

Table 2. Correlation analysis among the parameters tested.

Parameters tested	Moisture %	WHC %	pH	Light (LID)	C %	N %	Litter decomposition
Litter decomposition	0.242ns	0.080ns	-0.056ns	-0.304ns	0.194ns	0.116ns	1
Biomass carbon	0.584**	0.013ns	-0.198ns	-0.628**	0.566**	0.760**	0.506*
Gram + bacteria	0.330ns	0.014ns	-0.387ns	-0.446*	0.678**	0.633**	0.264ns
Gram – bacteria	0.211ns	0.204ns	-0.280ns	-0.205ns	0.852**	0.861**	0.096ns
Fungi	0.503**	0.138ns	-0.268ns	-0.066ns	0.817**	0.861**	0.109ns
Actinomycetes	0.699**	0.028ns	-0.367ns	-0.509**	0.808**	0.807**	0.167ns
Cellulolytics	0.342ns	0.186ns	-0.217ns	-0.033ns	0.680**	0.704**	0.410*
Amylolytics	0.448*	0.226ns	-0.245ns	-0.019ns	0.612**	0.656**	0.464*

** significant at $p<01$; * significant at $p<05$; ns not significant

Table 3. Summary of Analysis of variance (ANOVA) for all determined parameters.

Parameters tested	Sum of Squares	df	Mean square	F	Sig.
Moisture	59.16	2	29.58	1.97	.169 ns
WHC	3.69	2	1.85	0.61	.554 ns
pH	4.30	2	2.17	2.61	.101 ns
C	5.16	2	2.58	11.68	.001**
N	5.63	2	2.82	11.86	.001**
Biomass C	203829.80	2	101914.90	4.68	.023*
Gram+bacteria	10220.60	2	5110.30	17.47	.000**
Gram-bacteria	1212.64	2	606.32	13.22	.000**
Fungi	1583.71	2	781.85	8.66	.002**
Actinomycetes	5403.81	2	2701.90	25.77	.000**
Cellulolytics	268.93	2	134.46	5.74	.012*
Amylolytics	487.27	2	243.63	4.88	.020*
Litter decomposition	115.65	2	57.83	0.13	.877 ns
LID	18.04	2	9.02	25.95	.000**

** significant at $p<01$; * significant at $p<05$; ns not significant

Microbial Abundance

For all the six groups of microorganisms studied, UM showed the highest abundance, followed by P10, and then P6. The highest abundance was observed in spring and summer and the lowest was observed in winter. Great fluctuations were also observed and the lowest abundance was recorded in February 2004 (Figure 6, 7, 8, 9, 10, 11). ANOVA showed significant differences between study sites (Table 3) ($p<.01$). Tukey's test proved that the abundance of Gram positive and Gram negative bacteria and actinomycetes at P6 was not significantly different from P10, but P6 and P10 were significantly different from UM. The fungal, cellulase-producers' and the amylase producers' abundance at P6 did not differ significantly from P10, and P10 did not differ significantly from UM, but P6 did differ significantly from UM.

Table 2 (correlation analysis) shows the close correlation between microbial abundance and the physicochemical properties of the soil. The Gram positive bacteria were highly correlated with total carbon (r^2=0.678, p<0.01), and total nitrogen (r^2=0.633, p<0.01). The Gram negative bacteria showed close correlation with total carbon (r^2=0.852, p<0.01), and total nitrogen (r^2=0.861, p<0.01). Fungi was highly correlated with moisture content (r^2=0.503, p<0.01), total carbon (r^2=0.817, p<0.01), and total nitrogen (r^2=0.861, p<0.01). Actinomycetes proved to be highly correlated with moisture content (r^2=0.699, p<0.01), LID (r^2=0.509, p<0.01), total carbon (r^2=0.808, p<0.01), and total nitrogen (r^2=0.807, p<0.01). Cellulase-producers were closely correlated with total carbon (r^2=0.680, p<0.01), and total nitrogen (r^2=0.704, p<0.01). Amylase-producers showed close correlation with total carbon (r^2=0.612, p<0.01), and total nitrogen (r^2=0.656, p<0.01).

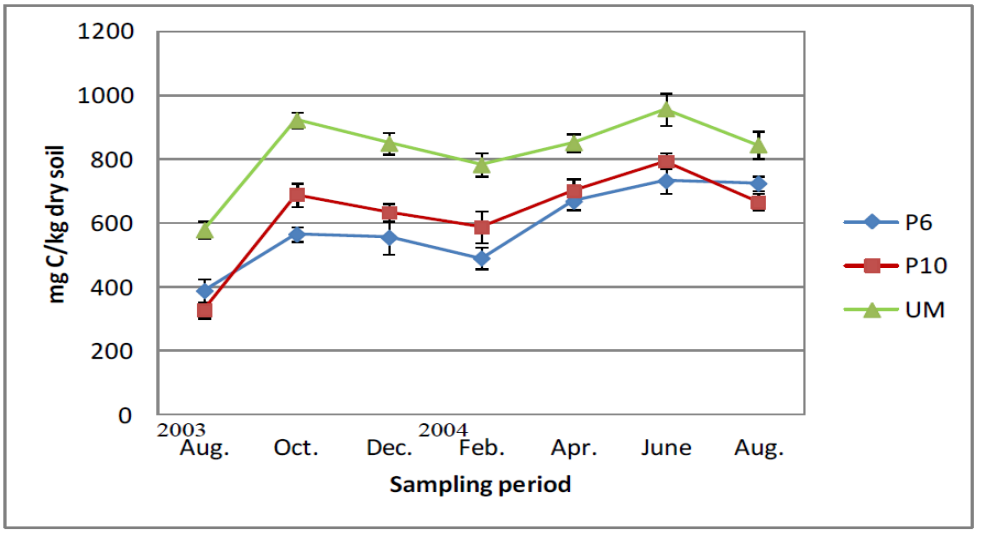

Figure 5. Average (±se) microbial biomass carbon at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

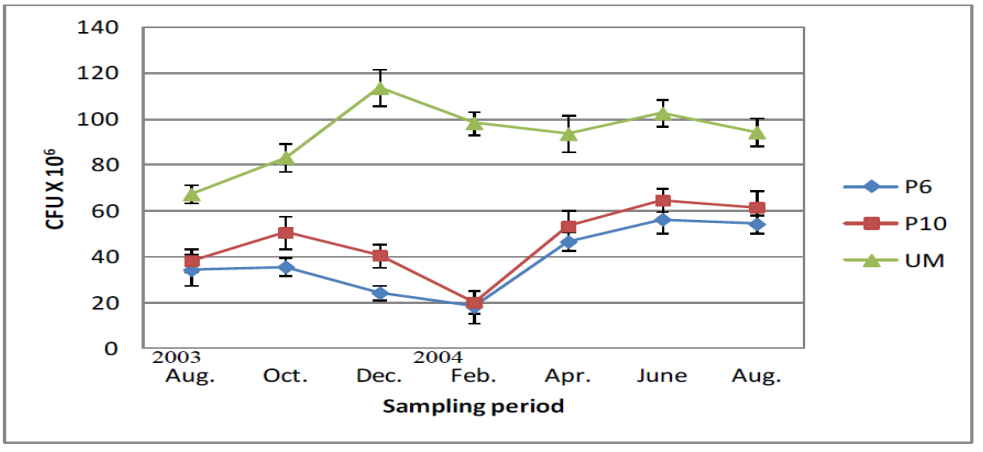

Figure 6. Average (±se) Gram positive bacterial abundance at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

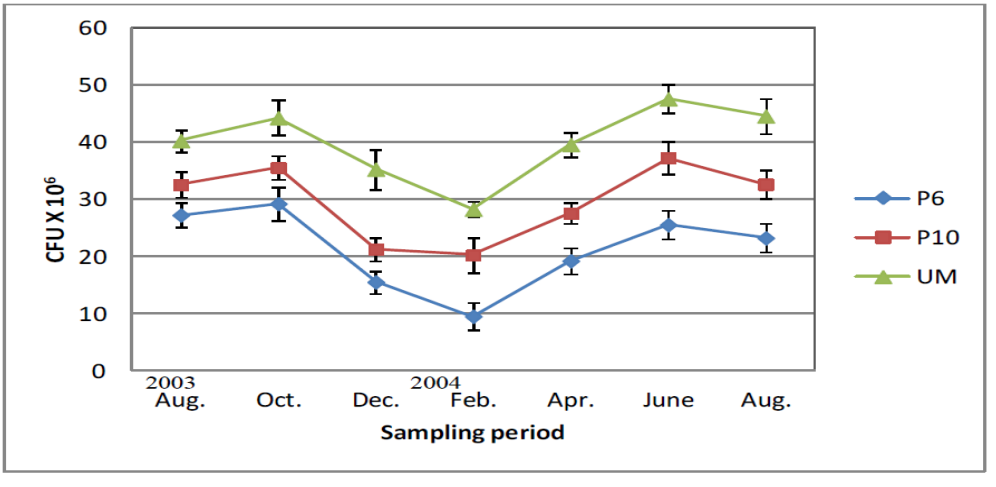

Figure 7. Average (±se) of the Gram negative bacterial abundance at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

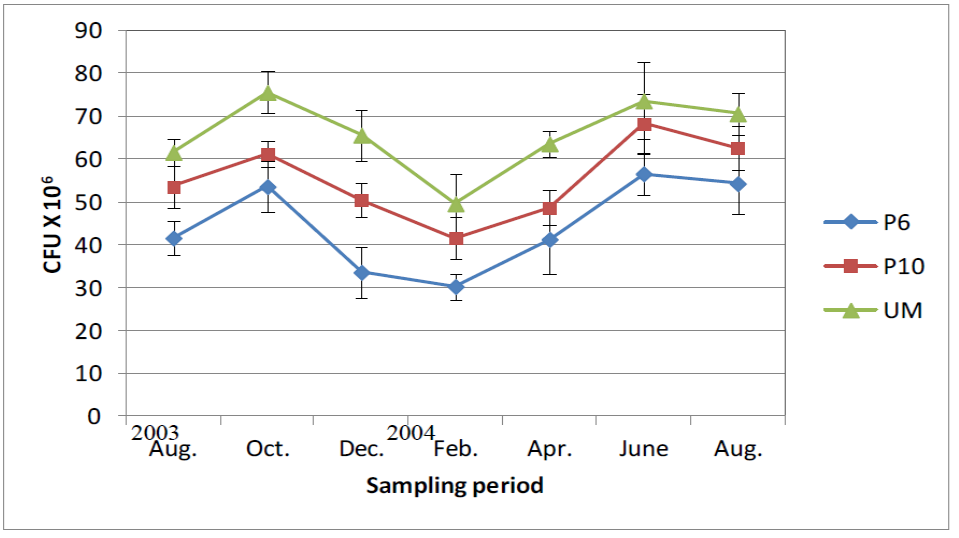

Figure 8. Average (±se) fungal abundance at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

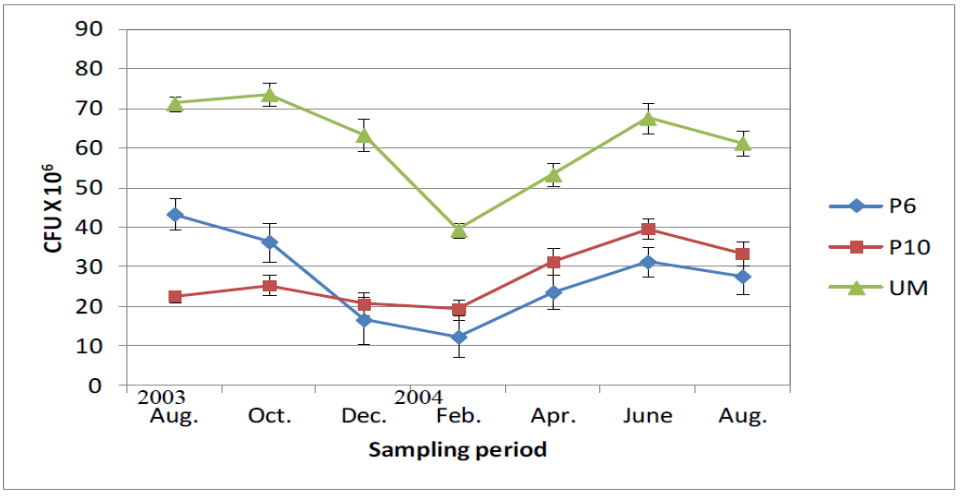

Figure 9. Average (±se) abundance of the actinomycetes at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

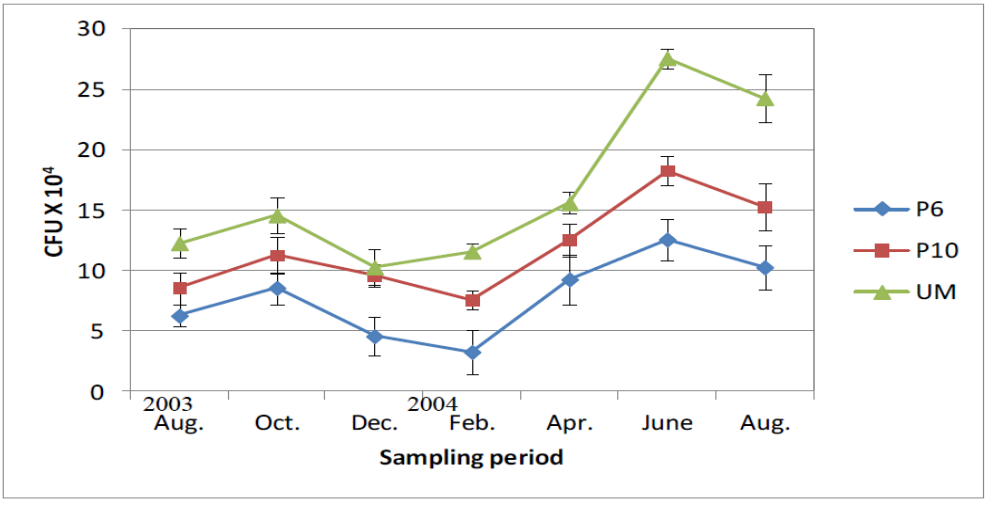

Figure 10. The average (±se) abundance of the cellulase-producers at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

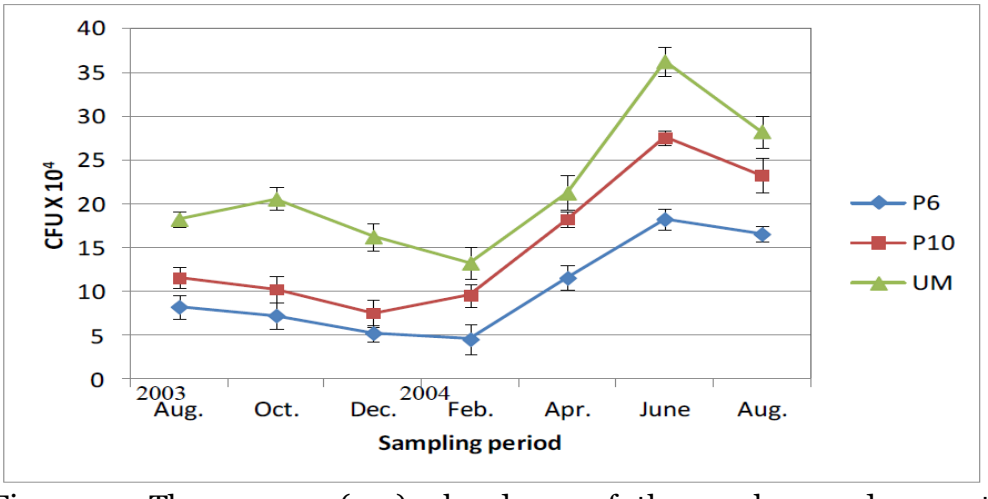

Figure 11. The average (±se) abundance of the amylase-producers at unmanaged forest without pine wilt disease (UM), 6-year old stand maintained by application of fertilizer and spraying of insecticide once a year, without pruning or thinning (P6), and 10-year old stand maintained by thinning (cutting of unwanted small diameter trees and under-storey vegetation) and pruning once a year (P10).

DISCUSSION

The physical characteristics (color, texture, moisture and water-holding capacity) of the three study sites did not differ significantly from each other. On the other hand, the chemical properties (pH, C and N) showed significant differences between sites. The high correlations among biological and physico-chemical properties of soil implied high interdependence among soil's characteristics. The unmanaged site (UM) showed C and N content two times higher than the two managed sites (P6 and P10). These results were in contrast to what Boerner and Sutherland (1997) had observed where thinned forest had greater mineral nutrients than the unmanaged plot. The pH in P6 and P10 were higher than UM by around 20% and 9% respectively. The slightly high pH in P6 was probably because of nitrogen fertilization (Guan 2016). The nitrification process (bacteria convert ammonium to nitrate) releases hydrogen ions (H+), which react with hydroxide ions (OH–) released by plants during the process of taking up of nitrate. The overall effect on soil pH is close to neutral (Guan 2016). The low C and N content can be attributed to the young age of the stand where most vegetation was still undergoing vegetative growth, needing higher nutrient allocation. P6 was a fast growing stand and since no thinning or pruning had yet been employed, competition for nutrients among vegetation was high and fertilization activity was not sufficient for the vegetative demands. In the case of P10, the thinning and pruning processes should have minimized the competition for available nutrients (Vesterdal et al. 1995), but because no fertilization was being done and the vegetation was still growing fast, nutrient pooling was not possible. In addition, the litter input decreased because thinned trees and cut branches were not left to decay but were gathered to prevent infestations. Thinning and pruning promote natural regeneration and expression of some inhibited understory vegetation (Zhu et al. 2003), thereby increasing nutrient consumption.

The rate of litter decomposition did not differ significantly between sites. There was also no indication of close correlation of litter decomposition to any of the physical, chemical and biochemical parameters measured in this study. The litter decomposition underwent different phases. This could be due to microbial communities which colonized leaf litter, underwent major temporal changes, both seasonally and over the course of litter decomposition (Voříškova and Baldrian 2013). Because of high light penetration to the forest floor after thinning and pruning, a faster rate of decomposition was expected, but this was proven to be not true. Although the thinned and pruned site showed the lowest mass remaining after 13 months of litter exposure, it was not significantly different from the other two sites. On the other hand, nitrogen fertilization was expected to increase the rate of decomposition by increasing microbial abundance and other decomposers like arthropods (Bird et al. 2004; Zhang et al. 2017), but this was also proven not to be the case in this study. If N limits microbial activity like decomposition (Kaye and Hart

1997; Hobbie and Vitousek 2000), then population and activity would increase after N addition, but this hypothesis was also not supported by the results of this study. Many studies have shown that N additions decreased or did not influence decomposition (Fog 1988; Aber 1998; Carreiro et al. 2000; Ågren et al. 2001).

Microbial biomass and microbial abundance were shown to be lower at the managed sites than at the unmanaged site. The stand type always has a strong influence on microbial biomass and abundance (Compton et al. 2004). Microbial biomass and the processes carried out by soil microorganisms depend upon complex interactions with plants (Singh et al. 1989; Bohlen et al. 2001; Zheng et al. 2017). Soil microorganisms are generally C limited (Wardle 1992) and, as a result, microbial biomass and abundance depend upon soil organic matter. In this study, the C and N content at the managed sites were a lot lower than that of the unmanaged site. This explains the low microbial biomass and abundance at managed sites. For P6, although nitrogen fertilization was being employed, the opposing effects of insecticide should also be considered. Because of the threat of pine wilt infestations, as the surrounding areas were still affected by the disease, this young stand was maintained by insecticide application to prevent infestation. Insecticide, on the other hand, is not just harmful to insects but its penetration to the soil kills microorganisms and other microflora and fauna (Aktar et al. 2009). Also, understory weeding was conducted, which made soil moisture evaporation increase and litter input decrease. With this, nutrient pools were also decreased. Although quite surprising, many studies have reported that nitrogen addition suppressed microbial biomass even in forest ecosystems where productivity is primarily N limited (Prescott et al. 1992; Smolander et al. 1994; Fahey et al. 1998; Scott et al. 1998; Colombo et al. 2016). For P10, because of thinning and pruning, gaps were created whereby light radiation towards the forest floor increased (Demarais et al. 2017). These gaps contributed to high air and soil temperatures in the forest microenvironment (Arunachalam and Arunachalam 2000). This kind of microclimate initially facilitated rapid decomposition on the forest floor (Arunachalam et al. 1996), but, because the litter input was decreased by thinning and pruning, decomposition of the organic layer happened instead. On the other hand, since P10 is in a hilly area, soil fertility may also be affected by heavy rainfall penetrating through the gaps, which may have caused erosion of top soil and leaching of organic matter.

There were seasonal variations in microbial biomass and the six groups of microorganisms studied. In all cases, high values were observed in autumn and spring. On the other hand, low values were observed in winter. These results are supported by the studies of Wüthrich et al. (2002) and Singh et al. (1989), who observed that microbial biomass is usually affected by season, with high amounts occurring in spring. On the other hand, this was in

contrast to the findings of Arunachalam and Arunachalam (2000), that microbial biomass peaks in winter.

It was proven by Tukey's test that the abundance of Gram positive and negative bacteria and actinomycetes at P6 was not significantly different from P10, but P6 and P10 were significantly different from UM. On the other hand, the fungal, cellulose-producers' and amylase producers' abundance at P6 did not differ significantly from P10, and P10 did not differ significantly from UM, but P6 differed significantly from UM. These relationships are a little complicated to explain just looking at the effects of the management practices on the microbial populations. This could probably be attributed to the conflicting results on the effects of N additions on microbial populations and their activities, and to the harmful effects of insecticides applied to prevent nematode contamination.

It was shown in this study that the sites maintained by thinning/pruning and nitrogen/insecticide applications had lower C and N content, and low microbial biomass and abundance than the unmanaged site. Similar results were observed by Zheng et al. (2017) where they observed that the effects of fertilization on microbial communities correlated with variations in pH, moisture and N availability. Despite these facts, it is difficult to conclude that all these results were due to the silvicultural practices employed at each site. The disturbance history has a strong relationship with the soil's current physical, chemical and microbial conditions. In one of the studies the authors conducted (Mabuhay and Nakagoshi 2012), it was observed that microbial biomass carbon was greater, and the rate litter decomposition was faster, in the site currently affected by pine wilt disease than in an unmanaged site without pine wilt disease. On the other hand, the abundance of Gram positive and negative bacteria, fungi, actinomycetes, and cellulase- and amylase-producers were low at the site affected by pine wilt disease. These general effects of pine wilt disease on the soil's properties may also determine the condition and responses of microorganisms to forest management practices following pine wilt disease. These negative impacts may have been enhanced by total deforestation and pest eradication prior to reforestation. This subject should be studied more in detail for better understanding and conclusion.

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