Biological activities of soils under rubber trees (Hevea brasiliensis) and interactions with trunk phloem necrosis

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RESUME

La qualité du sol influence fortement la productivité d'*Hevea brasiliensis* qui est un des plus grands producteurs de latex naturel. La nécrose de l'écorce de l'Hévéa (Trunk Phloem Necrosis : TPN) est la maladie de l'hévéa économiquement la plus importante or, à l'heure actuelle, son origine et les causes de son apparition sont mal connues bien que l'apparition de la TPN soit souvent liée à des sylvicultures réalisées dans des conditions extrêmes. Cette étude vise à préciser d'une part les relations entre les activités biologiques du sol et la présence de TPN, d'autre part à déterminer l'influence de différentes types de management du sol sur l'apparition de cette maladie.

Une étude a été menée sur une parcelle d'hévéa très infestée par la TPN. Des échantillons de sols et de faune ont été collectés sous les arbres malades (B), entre un arbre malade et un arbre sain (BH), sous un arbre sain (H) et entre deux arbres sains (HH). L'analyse de la faune du sol a montré que la présence de termites était fortement liée aux arbres malades.

Par ailleurs, la nécrose de l'hévéa semble interagir avec l'activité biologique du sol particulièrement pendant la saison des pluies. Ainsi, l'étude des activités enzymatiques du sol montre que les polysaccharidases (cellulase, xylanase et surtout amylase) sont plus faibles sous les arbres affectés par la TPN alors que les activités liées au cycle de l'azote y sont plus élevées.

Les communautés fongiques cultivables des différents types d'échantillons (B, BH, H, HH) ont été déterminées par culture sur milieu solide de Sabouraud. Bien que la densité fongique ne soit pas significativement différente entre les différentes zones, il apparaît que les espèces *Paecilomyces lilacinus* et *Trichoderma asperellum* sont majeures dans les sols prélevés à proximité des arbres malades (B et BH). Un dendrogramme de similarité réalisé à partir des séquences de 28S rDNA fongique montre que les communautés fongiques présentes sous les arbres sains sont très différentes de celles détectées dans les zones comportant des arbres infestés par la TPN. Ce résultat original doit faire l'objet d'études complémentaires afin de déterminer en suivant l'évolution de la maladie sur la parcelle, si les modifications de communautés fauniques ou fongiques observées entre les 4 zones (B, BH, H, HH) sont antérieures ou postérieures à l'apparition de la maladie et donc si elles pourraient constituer un élément de diagnostic de l'état sanitaire de l'exploitation afin de prévenir l'apparition de la TPN.

Une seconde étude s'est intéressée à l'influence de différents amendements organiques sur le fonctionnement biologique du sol sous hévéa ce qui devrait permettre en comparant les informations données par l'étude sur la plantation infestée de prévenir l'apparition de TPN. Les deux amendements comparés, apport de fertilisant organique et couverture de *Pueraria* + amendement organique, entraînent une augmentation de la croissance des arbres ainsi que de la production de latex, en comparaison avec une plantation sans fertilisation. Ces deux types d'amendements semblent aussi prévenir l'apparition de TPN.

Les échantillons de sols ont ici été prélevés dans les interlignes afin d'avoir la plus grande homogénéité possible entre les échantillons. Comme précédemment la macrofaune et les activités enzymatiques du sol ont été déterminées.

La densité de macrofaune du sol est significativement plus faible dans les parcelles sans amendement organique. Le groupe le plus abondant est constitué par les fourmis dont les populations sont beaucoup plus denses dans les zones ayant reçu un amendement organique

L'analyse en composante principale réalisée à partir des activités enzymatiques montre que la plupart des enzymes présentent des activités plus faibles dans les sols des parcelles sans amendement organique. Les sols des parcelles recouvertes de *Pueraria* ont les plus fortes activités xylanases alors que l'activité FDase est plus élevée dans les sols des parcelles n'ayant reçu que l'apport organique.

L'apport d'amendement organique en améliorant efficacement la teneur en matière organique du sol, non seulement permet un meilleur rendement en latex mais aussi en améliorant le fonctionnement biologique du sol prévient l'apparition de TPN.

ABSTRACT

Soil quality greatly sustains productivity of *Hevea brasiliensis*, which is the important source of natural latex production. The soil quality may have an influence in incidence of trunk phloem necrosis (TPN), which is the currently one of economically important disease of *H. brasiliensis*. This study aims to define the impact of TPN on soil biological activities and to investigate the impact of different soil management practices on soil functions. The TPN disease seems to have effects on soil biology, especially, in the rainy season. Principle component analysis showing associations between healthy and TPN trees, and soil enzyme activities revealed that polysaccharidase (cellulase, xylanase and, particularly, amylase) activities were lower under soil affected by TPN whereas *N*-acetyl-glucosaminidase activity was higher. Termite density was associated with soil under TPN areas. The method of culturable-dependant was used in order to analyze fungal populations. Although fungal density in soil under trees affected by TPN was not significantly different from soil under healthy trees, the fungi *Paecilomyces lilacinus* and *Trichoderma asperellum* were abundant in soil with TPN. The similarity dendrogram of 28S rDNA-DGGE (DNA was extracted directly from soil) revealed that soil fungal community in healthy tree zones was different from TPN tree zones. Soil biological activities in soils with different managements including without external organic input, external organic input and *Pueraria* cover crop + external organic input were investigated in order to find out the proper soil management for *H. brasiliensis*. Principal component analysis showing relationship between different soil managements and soil enzyme activites revealed that most enzyme activites were low in soil without organic management. Xylanase activity was the highest in soil with *Pueraria* cover crop + external organic input whereas the highest FDA hydrolysis activitie was asscociated with soil under external organic input. In investigating macrofauna showed that macrofauna density was the lowest in soil without organic amendment. Ant group was higher abundance in soil with organic amendments than soil without organic amendment. The productivity of *Hevea* tree including tree growth and latex yield was the highest in plantation with *Pueraria* cover crop +organic input, which was also the highest in organic matter and organic carbon contents in soil. This study observed that the soil with the lowest fertility, which indicated in soil without external organic input, seems to associate with high TPN incidence

THESIS OUTLINE AND OBJECTIVE

The main objectives of this study are i) to investigate impact of trunk phloem necrosis (TPN) disease of *H. brasiliensis* on soil biological activities and ii) to examine the influence of soil management with different organic practices on soil biological activities in *H. brasiliensis* plantation.

Soil biological activities in this study consist of macrofauna community, microbial community and enzyme activities.

In the chapter III.1, discriminations of soil biological activities between TPN affected trees and healthy trees were reported. This chapter reveals impact of TPN affected trees on soil biological activities. Some soil physical and chemical properties were also assessed to compare quality of soil between TPN affected trees and healthy trees. To understand the role of TPN disease on soil biology is an essential component of natural rubber ecosystem.

In the chapter III.2, impact of different soil management practices including soils without external organic input, with external organic input and with *Pueraria* cover crop + external organic input on soil physical and chemical properties, and soil biological activities was revealed. The selecting organic source is needed to ensure short-term productivity and to construct long-term soil quality.

The prospective outputs of this research are i) to obtain potentially biological indicators for impact of TPN affected tree on soil ecosystem. These indicators are useful to early detection of declining soil quality and ii) to obtain the database of soil managements on soil properties. This database contributes to provide the proper soil management practice, which is beneficial to improve soil quality. In addition, increasing soil fertility enhances plant productivity and TPN protection.

I - REVIEW PART

I.1 *Hevea brasiliensis* and trunk phloem necrosis

I.1.1 *H. brasiliensis* as economically important tree

Para rubber tree (*Hevea brasiliensis* Müll. Arg.) had been first found in Amazon basin. This tree is a member of family Euphorbiaceae which can produce natural latex (Purseglove, 1987). The latex is tapped by excision of external tissue of trunk (known as tapping) which contains laticifers (Rudall, 1987). This tree species is a perennial and humid tropical tree, the optimal conditions for growth of *H. brasiliensis* that are: the temperature is in range of 22-30 ^{o}C, relative humidity does not exceed 70 to 80 %, and annual rainfall is in between 1500 and 3000 mm. Furthermore, soil properties are also influence the growth and productivity of *H. brasiliensis*. Low latex yield was exhibited in soil under condition of acidic (pH range 4.37-4.54), sandy (75.6-82.6%). Additionally, the higher soil fertility strongly associated with higher latex yield (Akpan *et al.,* 2007). On the commercial plantation, *H. brasiliensis* take 5-10 years to reach maturity and the mature trees are 20-30 meters tall.

The *H. brasiliensis* is an economically important tree because its latex is the primary source of natural rubber. All of trees in family Euphorbiaceae, the natural rubber from *H. brasiliensis* is the most elasticity, resilience and toughness. These latex properties are efficient to use as raw materials of many products including transportation (e.g., tires and tire products), industrial (e.g., transmission and elevator belts, hoses and tubes, industrial lining, and bridge bearings), consumer (e.g., sport goods, erasers, foot wear and other apparel), and hygienic and medical (e.g., condoms, catheters and surgical gloves) sectors. The latex can be collected around 30 years or over depend on plantation management and tapping system. After the termination of latex production, the wood of this tree can be used in manufacture of furniture such as small boards, matches, packing boxes, compressed wood textiles and round arches, and also used for fuel.

There has been reported that the natural rubber production is the most in Asia, Africa and Latin American, respectively. During 2000-2006, the most natural rubber in the world was produced from Thailand (FAO, 2009). The *H. brasiliensis* is greatly important in socio-economic fabric of the Southern Thailand and nowadays, it rapidly extends to the Northeastern Thailand (which the soil quality is lower). However, currently, the main problem of latex production in Thailand is trunk phloem necrosis disease. This disease directly affects quantity and quality of latex.

I.1.2 Trunk phloem necrosis disease

The *H. brasiliensis* can be threatened by several pests and disease (which cause by biotic and abiotic factors), trunk phloem necrosis (TPN) is one of the most devastating in *H. brasiliensis* plantation worldwide (especially, industrial scale) because TPN disease is irreversible syndrome and can obstruct latex production. The TPN disease was first found in commercial plantation in Ivory Coast in 1980s by Nandris et al. (1991a). The first stage of this disease begins in the internal bark of trunk, this stage is difficult to detect by naked eye, and the phloem tissue containing the laticifers is necrotic. The investigation of phloem section of TPN affected tree by transmission electron microscopy revealed that cell walls, the middle lamella, plasmodesmata and membrane structures were disorganized (Nicole *et al.*, 1991). When accumulated necrosis was increased, the external bark is cracked, the cracking spread from collar towards the tapping panel. Furthermore, the high severity of TPN disease cause of incision drying up and discontinuing latex production (Nandris *et al.*, 1991a) (Figure 1).

The epidemiology pattern of TPN in the *H. brasiliensis* plantation seems to be contagious disease causing by biotic pathogen. Since the TPN was discovered, numerous of conventional and molecular etiological methods were used to diagnose the causal agents, however, no biotic causal agents involving fungi, bacteria, mycoplasm, virus and viroids were detected (Nandris *et al.*, 1991b; Pellegrin *et al.*, 2007). While, pathogen transmissions had been also investigated consisting bud grafting, bark implantation, tapping knife disinfection and spatio-temporal modeling of TPN dynamics, but no evidence revealed that the TPN disease can spread in plantation by transmission of pathogen (Pellegrin *et al.*, 2004; Peyrard *et al.*, 2006).

Because verification of TPN etiology cannot detect the biotic pathogens by present techniques, Nandris et al. (2004; 2005; 2006) who is the first breakthrough on TPN disease conducted that this disease may be caused by a combination of exogenous and endogenous stresses. The environmental stresses including soil compaction and soil fertility seem to be the major exogenous stresses for TPN disease apparition because soil compaction directly affects plant health by reducing soil porosity, soil water availability and plant root development. Meanwhile, low soil fertility directly influence plant physiological processes, this may reduce the resistance of TPN disease. However, endogenous stresses are also component of casual agent. Incompatibility of rootstock/scion can affect plant physiological strength. The tapping systems may, especially, high frequent tapping and over deep tapping

may also involve with plant physiological dysfunction. Disruption of cyanide metabolism may cause of cell death in TPN affected tree (Cherstin *et al.*, 2004). Hence, productivity of *H. brasiliensis* depends on soil quality level. The good soil quality may also induce resistance of *H. brasiliensis* to TPN disease.

Figure 1 Symptom of trunk phloem necrosis of rubber tree (*Hevea brasiliensis*)

I.2 Soil quality

I.2.1 Soil quality components

Soils are fundamental resource for plants and soil organisms. Most nutrients and carbon are held in the soil surface, especially, 5-10 cm of the soil profile, and hence this soil layer is important to regulate soil functions and processes (Sparling *et al.,* 2000). Soil quality is potential of soil to perform functions that are essential to plant, animal and human health. The definition of soil quality usually refers to three soil properties including physical chemical and biological properties (Table 1), these soil properties considerably influence plant growth development, plant available nutrients and reducing soil environmental stresses effect on plant (Elliott *et al.,* 1996).

Soil physical properties as refer to soil structural ability are the greatest important to soil quality because soil structure is the primitive factor of soil productivity. Soil textures consist of the relation of sand, silt and clay proportions are primarily important to plant development, soil organism activities and soil biogeochemistry cycling (Chiarini *et al.,* 1998; Silver *et al.,* 2000; Plante *et al.,* 2006). The soil textures also have effect on others soil physical processes. Increasing clay content within soil texture greatly influences soil aggregate stability (Schlecht-Pietsch *et al.,* 1994; Amezketa, 1999). Soil aggregation has positively associated with soil water holding-capacity, soil aeration, and soil infiltration (Mapa and Gunsena, 1995). Soil porosity property is an important part of soil physical quality because porosity levels can indicate the soil compaction which is measured by soil bulk density (Abu-Hamdeh, 2003). Increasing soil compaction levels can decrease quality of soil in respect to reduce infiltration, slow drainage, reduce aeration, restrict plant root development and increase topsoil erosion (Pabin *et al.,* 1998; Lipiec *et al.,* 2003; Neves *et al.,* 2003; Mandal and Tripathi, 2009). High soil compaction also adversely affects soil biological activities such as soil macrofauna community structure (Radford *et al.,* 2001) and microbial activities (Dick *et al.,* 1988; Torbert and Wood, 1992; Lee *et al.,* 1996; De Neve and Hofman, 2000; Jordan *et al.,* 2003). Hence, the good soil structure is fundamental to soil biological and chemical processes.

Soil chemical properties closely interact with soil physical and biological processes. Soil pH is a principal component of soil chemical properties because it strongly influences

available nutrients and activity of soil organisms (Skinner and Todd, 1998; Léonard *et al.*, 2004; Fuentes *et al.*, 2006). Moreover, pH levels in soil also affect soil physical process, especially, clay dispersion and flocculation (Amezketa, 1999). While, organic matter containing in soil is main source to supply energy for soil organisms. The organic matter decomposition processes result in releasing plant available nutrients which can be absorbed by plant (Chander *et al.*, 1998; Warren and Zou, 2002; Faterrigo *et al.*, 2006). Furthermore, soil organic matter can also enhance soil physical properties including soil aggregate stability, water holding capacity and soil bulk density. (Amezketa, 1999; Martens, 2000; Loveland and Webb, 2003; Tripathy and Singh, 2004). The cation exchange capacity (CEC) which is determination of cation absorbable and exchangeable capacity of soil particles is important for soil nutrient retention. The levels of CEC depend on soil texture characteristics and organic matter levels (Kaiser *et al.*, 2008). The CEC has benefit on soil chemical stabilization by binding nutrient-soil particle together, it is applied to be a tool for investigation of soil fertility an nutrient retention capacity (Yagi *et al.*, 2003; Suganya and Sivasamy, 2006; Vagen *et al.*, 2006). Additionally, salinity of soil which can be detected by electrical conductivity (EC) is a main constituent for disturbance of plant growth, soil microbial community and activities, and soil-water balance (Frankenberger and Bingham, 1982; Sinha *et al.*, 1986; Ramoliya *et al.*, 2004; Anjum *et al.*, 2005; Wong *et al.*, 2008). Hence, soil chemical mechanisms are a main factor for plant growth development and soil biological processes.

Soil biological properties have significantly influenced soil fertility and linked to soil physical and chemical properties. The biological indicators in respect of soil quality mostly assess the size and diversity of microbial biomass, soil respiration, available C and N, enzyme activity and macrofaunal biomass. Soil microbes and their activities have benefit on soil structure including soil erosion resistance and water infiltration, these properties positively affect plant growth (Gasperi-Mago and Troeh, 1979; Elliott *et al.*, 1996). Moreover, the soil microorganisms play the major role on soil nutrient cycling because they can degrade organic materials and convert the organic matter to available form for plant growth (McLatchey and Reddy, 1998; Ingram *et al.*, 2005; Schultz and Urban, 2008). Furthermore, soil respiration is used to assess competency of soil to sustain crop growth. Soil respiration is determined by measuring carbon dioxide production in soil surface which results from plant root, microbial decomposition of organic matter and faunal breathing. The rate of soil respiration is mostly regulated by organic matter levels, soil moisture, soil

temperature, and microbial activity (Buchmann, 2000; Epron *et al.,* 2004; Tang *et al.,* 2006). In addition, soil invertebrates are essential organisms to high-quality of soil because they can comminute and distribute material residues, and improve nutrient availability (Anderson and Ingram, 1993; Schädler and Brandl, 2005). The role of macrofaunal community can also enhance soil structure, soil stability, water infiltration and activities of soil microorganisms (Lavelle, 1988; Léonard *et al.,* 2004; Frouz *et al.,* 2006b).

Hence, numerous soil managements are created to improve the quality of soil for sustainable agriculture. At present, soil organic management is concentrated because it can reduce polluted chemical in soil and maintain long-term soil quality. However, efficiency of soil organic management depends on quality of organic matter. The different organic practices regulate soil fertility levels.

Table 1 Soil properties for characterizing soil quality

Physical	Chemical	Biological
Texture	pH	Microbial biomass
Infiltration	Salinity	Soil respiration
Water-holding capacity	Electrical conductivity	Labile organic carbon
Aggregate stability	Cation exchange capacity	Labile organic nitrogen
Soil bulk density	Organic matter	Key invertebrate
	Extractable, N, P, K	Earthworms
	Total organic C	

Source : (Elliott *et al.,* 1996)

I.2.2 Soil quality improvement

The goal of agriculturist is obtaining the good production which depends on quality of soil. Soil properties are important determinant for plant growth, crop productivity and sustainable agriculture (Warkentin, 1995). Several soil managements are created to maintain and improve soil health in all physical, chemical and biological properties. Additional, organic matters in the soil are the most important for soil quality improvement because organic carbon and nitrogen are the main source for plants and soil organisms. Furthermore, organic input can provide the short-term productivity and simultaneously, building long-term

soil quality. For commercial cropping system in Thailand, there are two main agricultural practices to improve soil efficiency namely organic amendments and cover cropping.

Organic amendments

Organic amendments are addition of organic matter sources into soil for modifying soil properties. Sources of organic materials include compost, crop residues, animal manure and green manure. Soil organic matter levels strongly affect soil physical, chemical and biological processes. The final product of organic matter decomposition is humus (or stable soil organic matter) which has benefit on soil physical properties. Soil organic matter levels can improve soil structure including aggregate stability, water infiltration and water holding capacity (Franzluebbers, 2002; Loveland and Webb, 2003; Lado *et al.*, 2004; Gajic *et al.*, 2006). The increasing ability of soil infiltration and permeability can also reduce excess salinity accumulation in soil (Miller *et al.*, 2005). Organic matter contributes to soil fertility because its decomposition processes have released available nutrients for plant growth such as nitrogen, phosphorus and potassium (Blair and Boland, 1978; Pinamonti, 1998; Ingram *et al.*, 2005; Lupwayi *et al.*, 2007; Mukuralinda *et al.*, 2009). In addition, organic matter can increase cation exchange capacity (CEC). This effect contribute to soil chemical stabilization by binding humic acid-mineral nutrients (Oorts *et al.*, 2003; Kaiser *et al.*, 2008). Furthermore, soil organic matter contents also enhance soil microbial community. Increasing microbial activities contribute to soil structure and directly affect soil fertility (Wander *et al.*, 1994; Nelson and Mele, 2006; Fliessbach *et al.*, 2007; Hamer *et al.*, 2008). Additionally, quality of organic materials strongly influence soil macrofaunal, the activity of macrofaunas enhance soil physical properties such as soil hydraulic capacity. Increasing macrofaunal biomass and diversity can contribute to soil microbial activity (Lal, 1988b; Mando, 1997; Mboukou-Kimbatsa *et al.*, 1998; Frouz *et al.*, 2006b; Frouz, 2008). Furthermore, organic matter can reduce soil polluted chemicals because humic acid can absorb chemical pollutants such as pesticides and herbicides (Senesi *et al.*, 1995; Beyer and Blume, 1996; Cells *et al.*, 1997).

Cover crops

Cover cropping or green manuring is agricultural practice by growing beneficial plants that can improve soil quality in agroecosystem. Several cover crops including grasses, small grains and legumes are useful to improve soil physical, chemical and biological properties. The leguminous plants are commonly used as cover crop to improve soil fertility

because they can provide nitrogen into soil by nitrogen fixation process which arises from rhizobial bacteria (Frioni *et al.,* 1998). Some cover crop species were efficient in recycling nutrients (Rosolem *et al.,* 2002; da Silva and Rosolem, 2003; Franchini *et al.,* 2004). When cover crops are plowed down or terminated, they release nutrients by mechanisms of decomposition and this also increase soil fertility. Because cover cropping is adding energy sources of living soil organisms, diversity and abundance of soil organisms are enhanced. Increasing activity of soil organisms positively influence soil quality (Boyer *et al.,* 1999; Cederbaum *et al.,* 2004; Ingels *et al.,* 2005; Blanchart *et al.,* 2006; Dinesh *et al.,* 2009). Furthermore, increasing organic matter levels by cover crop residues can improve soil physical properties such as soil structure, and water and nutrient holding capacity (Patrick *et al.,* 1957; Robertson *et al.,* 1991; Arevalo *et al.,* 1998; Muñoz-Carpena *et al.,* 2008). In addition, cover crop can protect damaging soil productivity by reducing or preventing soil surface from erosion (Greene *et al.,* 1994; Martinez-Raya *et al.,* 2006; Zuazo *et al.,* 2006; Zuazo and Pleguezuelo, 2008). The networks of cover crop roots are the suitable habitat for soil macrofaunas and also increase soil porosity (Carof *et al.,* 2007). Increasing soil porosity involves water infiltration and soil water storage (Joyce *et al.,* 2002). Cover crops can protect soil water evaporation and increase soil moisture (Hopkinson, 1971), this is important for activity of soil organisms. Additionally, cover crop can protect desired plant from pests and weeds. The growth of weeds and the germination of weed seeds are suppressed by thick cover crops (Bradshaw and Lanini, 1995; Linares *et al.,* 2008). Some cover crops have ability in destroying plant pests (know as trap crops) or attracting beneficial insects and predators of plant pests, these plant species can be used as biological control of plant pests (Bugg and Waddington, 1994; Rea *et al.,* 2002; Shelton and Badenes-Perez, 2006; Youn and Jung, 2008). Several cover crops can destroy plant disease cycle or reduce population of plant pathogens, and release toxic chemicals affecting phytopathogens in soil including bacteria, fungi and nematode (Crow *et al.,* 2001; Hartz *et al.,* 2005; Blanchart *et al.,* 2006; Timper *et al.,* 2006). Furthermore, cover crops can increase biomass of beneficial invertebrates and microorganisms in soil, which theirs activities can also reduce soil borne pathogens (Boyer *et al.,* 1999; Abawi and Widmer, 2000).

I.3 The role of soil macrofauna

Soil macrofaunal community plays important role on soil quality because its activities influence soil processes. Soil macrofaunal activities enhance soil physical properties including soil porosity and aggregate stability. These soil properties positively affect water infiltration, drainage, aeration and root growth development (Lavelle and Spain, 2001). Furthermore, soil chemical processes are also regulated by soil macrofaunal activity. The soil macrofaunas greatly associate with distribution and protection of organic matter, increasing available nutrients (Lavelle, 1988; Huhta *et al.,* 1998; Scullion and Malik, 2000; Warren and Zou, 2002). The soil macrofaunas are also important in neutralizing soil pH (Frouz *et al.,* 2006a). However, all of macrofaunal taxonomic groups (Anderson and Ingram, 1993), earthworms, termites and ants have been considered as soil ecosystem engineers because they strongly influence soil properties and provide the energy source for microorganism (Jouquet *et al.,* 2006). These soil invertebrates are important in soil physical properties including soil porosity, drainage, aeration and reducing bulk density (Lobry de Bruyn and Conacher, 1990; Mando *et al.,* 1996; Lavelle, 1997; Mando, 1997; VandenBygaart *et al.,* 2000). In addition, these macrofaunal species contribute to soil fertility because they play important role in nutrient turnover and soil nutrient cycling (Lobry de Bruyn and Conacher, 1990; Lavelle *et al.,* 1993; Mora *et al.,* 2005).

The earthworm burrows within soil can increase soil aeration, water infiltration (Pitkänen and Nuutinen, 1998). These soil properties can stimulate soil microbial activities. For instance, the nitrifying bacteria were higher in lining of earthworm burrow than in the soil outside burrow (Parkin and Berry, 1999). In addition, microorganisms living in guts of earthworms and termites can rapidly degrade components of organic matter such as cellulose and lignin (Rouland *et al.,* 1988; Rouland *et al.,* 1991; Zhang *et al.,* 1993; Rouland-Lefevre *et al.,* 2002; Hyodo *et al.,* 2003). This result can increase carbon source and mineral nutrients in soil. Likewise, the activities of earthworms, termites and ants can stimulate microbial activity (Scheu, 1987; 1990; Binet *et al.,* 1998; Holt, 1998; Daube and Wolters, 2000; Scullion and Malik, 2000; Chaou *et al.,* 2003; Desjadins *et al.,* 2003; Ndiaye *et al.,* 2004; Stadler *et al.,* 2006). Increasing microbial activities can contribute to soil structure and soil fertility.

In addition, the prominent ability of termites is clay transportation. Termites can transport clay particles from subsurface horizons to termitaria (Fall *et al.,* 2001; Jouquet *et al.,* 2002; Jouquet *et al.,* 2007). The stabilization of soil organic matter was influenced by binding mineral clay-organic matter (Wattel-Koek *et al.,* 2001). Likewise, soil clay can protect organic molecules by slow mineralization rate (Bosatta and Agren, 1997; Yang *et al.,* 2006). Some ants and termites are distributer of organic matter within ecosystem by

transporting organic matter over long distance (Wood, 1988; Anderson and Ingram, 1993). However, combining function of soil macrofauna and soil microorganism is important to maintain and to increase soil health.

I.4. Soil microorganisms

I.4.1. Importance of soil microorganisms

Soil microorganisms are greatly important in soil food web and natural equilibrium. Microorganisms in soil are significantly relative to healthy soil and healthy plant because they are a considerable component of soil physical and chemical processes. The soil microbial communities can improve soil structure for plant growth by enhancing soil aggregate stability. Bacterial polysaccharides and, fungal hypha and metabolic products play important role in binding soil particles together (Robertson *et al.,* 1991; Hu *et al.,* 1995; de Caire *et al.,* 1997; Caesar-TonThat and Cochran, 2000). Soil aggregation is necessary for soil quality to improve infiltration rate, water holding capacity and plant root development. Thus, decreasing microbial biomass and theirs activities are a result in reducing soil aggregation (Neves *et al.,* 2003; An *et al.,* 2009). Numerous species of microorganisms including protozoa, bacteria, fungi and nematode are contained in soil. However, bacteria and fungi seem to be the most important in soil nutrient cycling because they are the first organisms degrading organic materials as theirs energy source. Soil fertility is enhanced by increasing microbial biomass. Soil microbes strongly influence soil biogeochemical because they obviously express their ability in organic matter decomposition, nutrient mineralization and nutrient cycling (Barbhuiya *et al.,* 2004; Ingram *et al.,* 2005; Devi and Yadava, 2006; Faterrigo *et al.,* 2006; Fosu *et al.,* 2007; Lucas *et al.,* 2007). Furthermore, some soil microorganisms have potential to degrade or detoxify chemical pollutants and pesticides in soil (Reed *et al.,* 1989; Rhine *et al.,* 2003; Trabue *et al.,* 2006; Zhang *et al.,* 2008). However, structure of microbial community depend on many factors such as climate, moisture, topography, plant growth, and quantity and quality of substrates (Schinner, 1982; Alvarez *et al.,* 1995; Smith and Goodman, 1999; Norris *et al.,* 2002; Barbhuiya *et al.,* 2004; Tilak *et al.,* 2005; Devi and Yadava, 2006; Waldrop *et al.,* 2006). Additionally, soil chemical (e.g., pH and salinity) and physical condition (e.g., texture and soil-water potential) are also influence soil microbial efficiency (Zhang and Zak, 1998; Emmerling *et al.,* 2001; Gleeson *et al.,* 2008; Wong *et al.,* 2008; Sawada *et al.,* 2009). Hence, soil microbial community composition

structure can respond to environmental change and it has been used for monitoring impact of agricultural practices and ecological stresses on soil health (Steenwerth *et al.,* 2002; Potthoff *et al.,* 2006).

I.4.2. Analysis of soil microbial community structure

The reservoir of soil microorganisms is the pore structure within and between soil particles. The community of soil microorganisms can use as useful indicator of soil quality and ecological stresses because of theirs adaptation. Because soil microbes are greatly important in agroecosystem, the microbial community structure assessment with accurate and reliable methodology is necessary. The methods for studying soil microbial diversity can be categorized into two major groups: biochemical-based and molecular-based methods (Kirk *et al.,* 2004).

Plate count is one of common methods that used for analyzing soil bacterial and fungal diversity. This method is a biochemical-based method to enumerate the viable soil microorganisms. The viable count has benefit to provide information on the active, heterotrophic component of population. This method has been used to evaluate the response of soil microorganisms to polluted soil (Thompson *et al.,* 1998; Piotrowska-Seget *et al.,* 2005; Oliveira and Pampulha, 2006) and agricultural practices (Buyer and Kaufman, 1997; Willison *et al.,* 1997; Leckie *et al.,* 2004; Zablotowicz *et al.,* 2007; Zheng *et al.,* 2007). However, many bacterial and fungal species in soil cannot be cultured in the present methods under laboratory condition (Torsvik *et al.,* 1990; Torsvik *et al.,* 1998; van Elsas *et al.,* 2000). The molecular-based methods have been developed to study microbial diversity in natural environments because these methods can detect non-culturable microorganisms. The denaturing gradient gel electrophoresis (DGGE) which is one of PCR-based approaches is mostly used to evaluate microbial community of environmental sample. Because the PCR products of environmental sample consisting the similar DNA sizes cannot be separated by conventional separation using agarose gel electrophoresis, these DNA can separate and migrate through a polyacrylamide gel by DGGE technique. Furthermore, the DGGE can rapidly analyze the large number of samples and is also reliable and reproducible (Lerner *et al.,* 2006; Nakatsu, 2007; Liang *et al.,* 2008). This approach has been diversely used to analyzed soil microbial community such as impact of perturbed agricultural soil (Jensen *et al.,* 1998; Øvreås *et al.,* 1998), agricultural practices (Nakatsu *et al.,* 2000; Vepsäläinen *et al.,* 2004; Stark *et al.,* 2007), fumigants (Ibekwe *et al.,* 2001), land usage (Bossio *et al.,* 2005),

thermal gradient (Norris *et al.,* 2002) and plant root exudation (Yang and Crowley, 2000) on soil microbial diversity. However, the accuracy of microbial community investigation can be increased by using a combination of culture-dependent and culture-independent methods (Ellis *et al.,* 2003; Edenborn and Sexstone, 2007; Fang *et al.,* 2007). Furthermore, using soil microbial community structure combining soil enzyme activity are usually used to assess impact of soil environment change on soil health arising from polluted chemical (Akmal *et al.,* 2005; Martínez-Iñigo *et al.,* 2009) and soil managements (Sun *et al.,* 2004; Stark *et al.,* 2007; Stark *et al.,* 2008) because large numbers of soil samples are rapidly investigated and it reveal the real impact.

I.5. Enzyme activity in soil

All biochemical transformations in soil are related to the presence of enzymes which are produced by microorganisms (de Caire *et al.,* 2000; Stark *et al.,* 2008), soil animals and plants (Gramss *et al.,* 1999). Nevertheless, the most of enzymes in soil are originated from microbes because they have larger biomass, higher metabolic activity and larger amount of extracellular than plants and animals. Enzymatic mechanism contributes to soil health, which is important for plant productivity. Soil enzymes are the great importance for agriculture because they are involved in soil fertility (Dick *et al.,* 1988; Bandick and Dick, 1999; Hu and Cao, 2007). Organic materials are degraded and transformed into available forms by mechanism of soil enzyme. Soil enzymes play specific role in soil cycling of nutrients such as nitrogen, carbon, phosphorus and sulphur (Gianfreda *et al.,* 2005; Acosta-Martínez *et al.,* 2007; Chen *et al.,* 2008; Sardans *et al.,* 2008). The evaluation of integrative activity of several soil enzymes is greatly efficient to predict the quality of soil because soil enzyme activity is closely related to soil physical and chemical properties, and soil microbial community (Decker *et al.,* 1999; Andersson *et al.,* 2004; Roldán *et al.,* 2005; Acosta-Martínez *et al.,* 2007; Iovieno *et al.,* 2009). Furthermore, some toxic chemicals including pesticides and heavy metals are degraded or detoxified by soil enzyme (Gibson and Burns, 1977; Burns and Edwards, 1979; Niemi *et al.,* 2009). However, the activity of enzyme depends on soil texture, temperature, pH, moisture, heavy-metal contamination, quality and quantity of substrates (Sinsabaugh and Linkins, 1987; Virzo De Santo *et al.,* 1993; Rutigliano *et al.,* 1996; Fioretto *et al.,* 2000; Kourtev *et al.,* 2002; Hinojosa *et al.,* 2004; Sardans and Peñuelas, 2005; Acosta-Martínez *et al.,* 2007).

The activity of soil enzymes is more sensitive than other soil properties (Ndiaye *et al.,* 2000) and rapidly responds to soil environmental change due to agricultural practices and cropping systems (Bandick and Dick, 1999; Kandeler *et al.,* 1999; Klose *et al.,* 1999; Acosta-Martínez and Tabatabai, 2001; Badiane *et al.,* 2001; Hinojosa *et al.,* 2004; Hu and Cao, 2007; Iovieno *et al.,* 2009; Jin *et al.,* 2009). In biochemical analysis, using soil enzyme activity to assess soil quality seems to be elementary whereas obtained results are correlated to other soil properties (Klose *et al.,* 1999; Ndiaye *et al.,* 2000). Hence, several soil enzyme activities are useful to early detection of soil quality change and they can provide information on soil managements or soil ecological stress.

I.6 Soil ecological stress

Soil ecological stress is a result from soil environmental stresses. The stresses of soil environment including abiotic and biotic factors can disturb soil physiological, chemical and biological functions. However, capacity of soil ecosystem to resistant environment stress was limited by thresholds of its resistance. When the strength of these stresses was increasingly, soil ecological changes are caused and declining soil quality is resulted. In addition, the environmental stress is considered that as the great damage of plants because it affects plant physiological functions and plant cell death is consequent.

The environmental stress can classify to several categories arising from both natural and artificial factors such as physical stress, wildfire, pollution, radiation, climatic stress and biological stress. The biological stress can be resulted from soil managements and diseases and hence, this stress may significantly affect on soil agricultural ecosystem.

Tillage

Tillage is an agricultural practice which directly affect on soil physical and chemical properties (Hulugalle *et al.,* 1997). Tillage practice can obstruct process of soil aggregate and is primary cause of soil ecological deterioration over long term (Horn *et al.,* 2003; Williams, 2004). Soil physical properties which affect soil organisms such as water content, soil temperature, soil aeration and mixing of crop residue within soil matrix were change (Kladivko, 2001; Clermont-Dauphin *et al.,* 2004). Soil organic matter in 0-10 cm deep layers which benefit soil microbes and macrofauna was decreased by ploughing (Clermont-Dauphin *et al.,* 2004).

The conventional tillage have reduced soil macrofaunal abundance (Neave and Fox, 1998), especially, earthworms which greatly influence on soil properties such as soil nutrient cycling, soil fertility and soil aggregate (Lavelle, 1988; Lavelle and Spain, 2001). Because the stability of soil aggregates is deteriorated by conventional tillage, the soil compaction is consequence (Shakir and Dindal, 1997). Moreover, the furrows within soil which originate by soil organisms have been also destroyed.

Agricultural soil under reduced tillage and no tillage have associated with higher microbial biomass carbon and microbial biomass nitrogen which are important in increasing soil organic matter content, N mineralization in soil and nutrient supply to crops (Wright *et al.,* 2005). Hence, tillage conservation can maintain soil physical, chemical and biological properties (Reeves, 1997; Amezketa, 1999; Shukla *et al.,* 2003).

Monocropping

Monocropping system or continuous cultivation is the agricultural practice which planting the same crop year after year, without practicing crop rotation or resting soil. Monocropping system may have negative impacts on long-term productivity due to decreasing soil quality. Some plant monocultures decreased soil aggregate stability, soil organic matter, available nutrients and cation exchange capacity, while acid saturation was increased (Dalal and Mayer, 1987; Ewel *et al.,* 1991; Amezketa, 1999; Buschiazzo *et al.,* 1999). The lower soil microbial diversity and biomass were detected in the longer monoculture (Holt and Mayer, 1998; Kaur *et al.,* 2000; Yao *et al.,* 2006; Wu and Wang, 2007), the lowering of soil microbes may associate with reducing soil enzyme involving nutrient cycling (Gajda and Martyniuk, 2005). Soil macrofaunal community including biomass, density and diversity was also decreased in long-term continuous planting (Lal, 1988a; Lavelle, 1988; Dangerfield, 1990; Decaëns *et al.,* 1994; Schmidt *et al.,* 2001; Giller *et al.,* 2005; Sileshi and Mafongoya, 2006). These biological effects can cause of deterioration of soil physical and chemical properties in the future, and which consequently led to decline of plant productivity.

Soil salinity and sodicity

Soil salinity and sodicity are soil which contains excessive accumulation of salts. Excess salts in soil cause deterioration of soil quality and agricultural productivity. Although soluble salinity positively associates with soil aggregate stability, high salinity can reduce plant growth, plant yield and quality of plant products (Verma and Neue, 1984; Abdul-Halim

et al., 1988; Marosz, 2004; Ramoliya *et al.,* 2004; Ramoliya *et al.,* 2006; Dai *et al.,* 2009). While, high sodium concentrations or high sodicity can disrupt soil physical processes including decreasing infiltration, decreasing hydraulic conductivity and surface crusting (Frenkel *et al.,* 1978; Agassi *et al.,* 1981; Painuli and Abrol, 1986; Mamedov *et al.,* 2001; Levy *et al.,* 2005). However, both salinity and sodicity have adversely affected soil microbial community and biogeochemical processes (Frankenberger and Bingham, 1982; Rietz and Haynes, 2003; Sardinha *et al.,* 2003; Tripathi *et al.,* 2006; Wichern *et al.,* 2006; Muhammad *et al.,* 2008; Wong *et al.,* 2008).

II. MATERIALS AND METHODS

II.1 Experimental site description

The experimental site was the rubber (*H. brasiliensis*) plantation in Buri Rum province, northeastern Thailand (15° 16′ 18″ N and 103° 0′ 6″ E). This region is tropical climate. The rainy season is from May to October and the dry season is November through March. During the 5 year period 2001-2006, the mean annual temperature for this area was 26 ^{o}C and annual precipitation was 1207 mm (which is very low for rubber plantation). Three experimental sites were selected and the description of each site was showed in table 7. All the sites were sampled during the rainy season (July to October 2006) and dry season (December to February 2008).

For chapter III.1, the *H. brasiliensis* plantation with high incidence of TPN disease (20-25% of trees affected) was selected. The *H. brasiliensis* was planted in 1997. Before planting *H. brasiliensis*, the sugarcane (*Saccharum* sp.) was planted in this area. This plantation characterized by low input farming.

For chapter III.2, three *Hevea* plantations were selected by depending different soil managements as follows: no organic amendment (S1) and amending with external organic input (S2) and amending with *Pueraria* cover crop + organic input (S3). The description of each site showed in table 7.

II.2 Macrofauna sampling

In each sampling zones, macrofauna in soil and litter were collected to analyze density and diversity. The macrofauna was defined as the invertebrates with body length greater than 2 millimeters. Litter macrofauna was sampled in an area of 2 x 2 meters. The litter was rapidly put into plastic bag and then macrofauna was carefully collected by hand. Soil macrofauna was performed according to the Tropical Soil Biology and Fertility (TSBF) method (Anderson and Ingram, 1993). The block of soil (25 x 25 x 30 cm) within middle of litter macrofaunal sampling area was taken and macrofauna was collected by hand at three depths (0-10 cm, 10-20 cm and 20-30 cm). The collected macrofauna was preserved in 70% alcohol, except earthworm was kept in 4% formaldehyde. The invertebrates were determined taxonomic groups under standard laboratory.

II.3 Soil sampling and preparation

In each sampling zone, a block of soil (12 x 12 x 10 cm) adjoining zone of litter macrofaunal sampling was taken using hand trowel. The soil block was separated into three layers (0-2 cm, 2-5 cm and 5-10 cm). In each depth, sample soil was air-dried and ground to pass through a 2 mm sieve. Plant residues and roots were removed.

II.4 Soil analysis

Prepared soil sample was used to analyze some soil properties. Soil pH was measured by pH meter using soil : water ratio of 1:1. Organic carbon was measured by oxidizing the soil with potassium dichromate and concentrated sulphuric acid and the remaining concentration of dichromate and ferrous ions determined by titration. The total N was measured by micro kjeldahl method. The organic matter was determined by Walkley and Black method using wet oxidation. Cations exchange capacity (CEC) was determined using double acid (Boric acid and sulphuric acid). Soil texture was performed by hydrometer method.

II.5 Soil enzyme activity

The soil sample with purified water (1 g/150 µl) were incubated at 25 ^{o}C for 48 h to reactive the microflora. The activity of β-glucosidase *(EC* 3.2.1.21), *N*- acetyl- β-D-glucosaminidase (EC 3.2.1.30), amylase (EC 3.2.1.1), cellulase (*EC* 3.2.1.4) and xylanase (EC 3.2.1.8) were assayed by a modified procedure described by Mora (2005). The modified Berthelot reaction (Kandeler, 1996) was used to measure urease (EC 3.5.1.5) activity. Total microbial activity potential was measured through Fluorescein diacetate (FDA) hydrolysis assay, which hydrolyze colorless fluorescein diacetate and release a colored end product fluorescein (Green *et al.*, 2006). The enzyme assays were carried out in triplicate to measure the activities and Control was simultaneously performed in each sample

Heterosidases

β-glucosidase and N-acetyl-β-D-glucosaminidase activities were assayed using *p*-nitrophenyl (PNP)-β-D-glucopyranoside and *p*-nitrophenyl (PNP) N-acetyl-β-D-

glucosaminide (Sigma) as substrates. Soil (200 mg) was incubated at 37 ^{o}C for 2 hours with 200 µl of 1% (w/v) substrate and 100 µl of citrate phosphate buffer at pH 5.8 (McIlvaine, 1921). After incubation, the sample was centrifuged at 13000 rpm for 5 min and the supernatant (250 µl) was placed into spectrophotometer tube (size 5 ml). The reaction was stopped by adding 3 ml of 2% Na_2CO_3 (w/v). The absorbance was measured at 420 nm and the enzyme activity was expressed as µg PNP g^{-1} h^{-1} by comparison with standard curve.

Polysaccharidases

The amylase, cellulase and xylanase activities were determined using starch, carboxymethyl-cellulose (CMC) and xylan as substrates. Soil (200 mg) was incubated with 200 µl of 1% (w/v) substrate and 100 µl of citrate phosphate buffer at pH 5.8 at 37 ^{o}C for 4 hours with constant agitation. After incubation, sample was centrifuged at 13000 rpm for 5 min and 250 µl of supernatant was placed into glass tube. The reducing sugar was measured using the method described by (Somogyi, 1945) and (Nelson, 1944). 500 µl of Somogyi solution (diluted with purified water, 1:1) was added and boiled for 20 min. The sample solution was immediately cooled using laboratory water. Afterwards, 250 µl of Nelson solution was added and gently vortexed. Purified water (4 ml) was added and the absorbance was measured at 650 nm. The result was expressed as µg glucose g^{-1} h^{-1} by comparison with standard curve.

Urease

The urease activity was measured by determination of released ammonium. Soil sample (200 mg) was mixed with 400 µl of 100 mM borate buffer (pH 10.0) and 50 µl of 720 mM urea. After incubation at 37 ^{o}C for 4 hours with constant agitation, the reaction was stopped with 3 ml of acidified 2 M KCl and 1.5 ml of the mixture was centrifuged at 13000 rpm for 5 minutes. The supernatant (1 ml) was mixed with 5 ml of a 1:1:1 (v/v/v) mixture of sodium salicylate/ sodium nitroprusiate reagent, 0.3 M NaOH and distilled water. Afterwards, 2 ml of 39.1 mM sodium dichloroisocyanurate and 9 ml of distilled water were added. After agitation in the dark for 30 minutes, the absorbance was measured at 660 nm and the enzyme activity was express as µg N-NH_4^+ g^{-1} h^{-1} by comparison with standard curve.

Fluorescein diacetate (FDA) hydrolysis

Soil (200 mg) was mixed with 25 µl of 4.8 mM FDA solution and 1.5 ml of a citrate phosphate buffer at pH 5.8. The sample was incubated at 37 ^{o}C for 2 h with constant

agitation. After incubation, 25 µl of substrate was added to control. The suspension was centrifuged at 13000 rpm at 4 ^{o}C for 5 min and 750 µl of supernatant was taken. The activity was stopped by adding 750 µl of acetone. The amount of FDA hydrolyzed was measured as absorbance at 490 nm and the activity was expressed as µg fluorescein g^{-1} h^{-1} by comparison with standard curve.

II.6 Soil fungal community

III.6.1 Soil fungal enumeration and isolation

Enumeration of soil fungi were conducted according to a method adapted from Diouf et al. (2005) using plating dilution method. Soil (5g) was agitated in 50 ml of dispersing solution for 30 min and allowing soil precipitation for 15 min. The fungal suspensions were decimal diluted in physiological solution (9g/1 of Nacl). Each of fungal dilution with 200 µl was cultured in Sabouraud medium (added 0.005% w/v of Chloramphemicol – Sigma). The assay was performed in triplicate. The numbers of fungal colonies were determined after incubation at 27 °C for 5 days. The differential colony morphotypes were isolated and purified on Sabouraud medium. The mycelia of selected fungi were conserved at -20 °C for molecular identification.

III.6.2 Identification of culturable fungi

The selected fungi were identified the taxonomic position by using molecular biology technique. DNA was extracted from mycelium according to Di Battista method (Di Battista, 1997). The nucleotide fragment of 650 bp (ITS1-5.8S-ITS2 region) was amplified by PCR technique using primer set ITS1 (5'TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White *et al.,* 1990). PCR amplification was performed using Ready-To-Go Taq poloymerease (Pharmacia). The condition of PCR cycles were initial denaturation at 94 °C for 5 min, 29 cycles of 94 °C for 45s, 60 °C for 45s, 72 °C for 1 min and final elongation step at 72 °C for 30s. PCR amplification was performed with a thermal cycle (GenAmp PCR System 2400; Perkin-Elmer). All PCR products were investigated using 5 µl of product by electrophoresis in 2% (w/v) agarose gel in 0.5X TAE buffer (Biorad) and ethidium bromide (0.5 µg/ml) staining. DNA sequencing was performed by Genome Express

(Grenoble, France). The DNA sequences were compared with other DNA sequences using GenBank BLAST searches.

III.6.3 Genetic diversity of soil fungi

Fungal DNA was extracted from soil samples as described by Porteus (Porteous *et al.,* 1997). The DNA region of 28S rDNA was amplified using primer set 403f (5' GTGAAATTGTTGAAAGGGAA 3') and 662r (5' GACTCCTTGGTCCGTGTT 3') with 40 bases GC clamp (Sigler and Turcob, 2002). PCR amplification was performed using Ready-To-Go Taq poloymerease (Pharmacia). Cycling condition were initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30s, 50 °C for 1 min, 72 °C for 2 min and final elongation step at 72 °C for 10 min. PCR amplification was performed with a thermal cycle (GenAmp PCR System 2400; Perkin-Elmer). The PCR products were investigated as described above and PCR amplicons were determined for different position by DGGE (Denaturing Gradient Gel Electrophoresis) using DcodeTM Universal Mutation Detection System (Biorad, Richmond, CA). For DGGE, 20 µl of product was loaded onto 8% (w/v) polyacrelamide gel containing linear gradient of the denaturants urea and formamide increasing from 35%-60% (100% denaturant contains 7 M urea and 40% (v/v) formaminde). Gel electrophoresis was carried out at initial 20 V for 10 min then 75 V for 16 h. Gels were run in 0.5X TAE at constant temperature of 60 °C. Gels were stained in an ethidium bromide solution and photographed under UV transillumination using GelDoc 2000 system (Biorad).

II.7 Analysis of plant growth

In each sampling zone, four trees surrounding the sampling zone, perimeter in each tree at height of 150 cm from base was measured for assessment of tree growth.

II.8 Statistical analysis

The averages of tested parameters in different zones were compared and statistic difference was measured by ANOVA-Tukey test ($P<0.05$) using R program version 2.7.0. The relationship between enzyme activities and sampling zones was determined by principal component analysis (PCA) using ADE-4 software (Thioulouse *et al.,* 1996). A permutation test was used to check the significance of the groups suggested by PCA.

For 28S rDNA diversity, the DGGE banding patterns were analyzed based on the band appearance and disappearance using image analysis software Quantity One (Bio-rad). Banding data of DGGE gel were used to evaluate genetic similarity of fungi among different four sampling zones. The genetic similarity of fungal 28S rDNA was measured using Dice coefficients and similarity dendrogram was created using UPGMA (Unweighted Pair Group Method of Arithmetic means) through the program, Quantity One version 4.2.1.

III - RESULTS

CHAPTER III.1

TPN AND SOIL ENVIRONMENT

Abstract

Trunk phloem necrosis (TPN) is currently one of the most economically important diseases of the *Hevea brasiliensis*. Investigation for etiology TPN revealed that no biotic pathogen was detected, however, a multidisciplinary study has recently suggested that the disease may be caused by a combination of exogenous and endogenous stresses. These stresses can affect plat physiology and may also have an incidence on soil biology and soil biochemistry. In this study, macrofauna diversity, soil enzyme activities, soil microbial community and soil chemical properties were compared for the soils under trees affected by TPN and under healthy trees. The impact of TPN on soil biological activities seems to be expressed in rainy season because maybe the trees were continuously stressed by tapping. The macrofauna analysis revealed that termite density in both litter and soil was the highest in areas with TPN affected trees. Earthworm and ant abundances were high in soil under healthy trees. Coleoptera abundance and diversity were low in areas with TPN affected trees. The principal component analysis revealed that polysaccharidase (cellulase, xylanase and, particularly, amylase) activities were lower in soil under trees affected by TPN whereas N-acetyl-glucosaminidase activity was higher. Investigating fungal density in soil showed that *Paecilomyces lilacinus* and *Trichoderma asperellum* were dominated in soil with TPN. The similar dendrogram of 28S rDNA-DGGE banding patterns for examining fungal community composition showed that the community of fungi under TPN was clearly divided from under healthy tree. These findings suggested that termite density and N-acetyl-glucosaminidase activity are useful as indicators of TPN, while lower values of polysaccharidase activities in TPN areas are the consequences of this disease. The impact of TPN on soil ecosystem was discussed.

III.1.1 INTRODUCTION

Hevea brasiliensis (well know as para rubber tree) is an important source of natural. Trunk phloem necrosis had become a serious problem in *H. brasiliensis* plantation system because this disease prevents latex production. TPN was first described in the 1980s in Ivory Cote by (Nandris *et al.,* 1991a). The first visible symptoms are the necrosis of the bark at the collar with thin cracks that extend upward towards the tapping panel. When the inner phloem, which contains lacticifers, becomes affected, the latex production is affected and the tapping panel and incision dry up (Nicole *et al.,* 1991). On-going and completed studies to identify a biotic causal agent have failed to confirm any biotic causal agents such as fungi, bacteria, mycoplasma, virus or viroids (Nandris *et al.,* 1991b; Pellegrin *et al.*, 2004; Pellegrin *et al.,* 2007). In addition, several experiments (bud grafting, bark implantation, tapping knife disinfection) and the spatio-temporal modeling of TPN dynamics have shown no evidence of pathogen (Peyrard *et al.,* 2006).

The first breakthrough on TPN was reported by (Nandris *et al.,* 2004; 2005; Nandris *et al.,* 2006) who concluded that this disease may be caused by a combination of exogenous and endogenous stresses. In particular, soil compaction which can induce water stress affecting the health of the trees and low soil fertility were thought to increase the risk of the disease. Furthermore, the non-optimal vascular interface between the rootstock and scion, combined with a major physiological dysfunction (cell disruption) can lead to the release of the native cyanide in the inner bark, thus causing the death of the cells and the spread of the necrosis in the phloem (Cherstin *et al.,* 2004). All these stresses may disrupt physiological metabolisms of rubber trees and consequently affect the soil biological microenvironment around the tree. However, the real impact of TPN on soil ecology is unknow.

Soil is a basic resource for agricultural production systems. Soil quality is fundamental to the success of crop production systems. Soil quality is associated with soil biological activity. Soil macrofauna, considered to be ecological engineers or ecosystem engineers, play an important role in soil formation, affecting organic matter decomposition and nutrient cycling. Increasing soil macrofauna diversity tends to increase the density of soil microorganisms as macrofauna can modify the conditions for microorganisms which are important sources of enzymes in soil (Mora *et al.,* 2005). Enzymes are the main mediators of soil biological process: organic matter degradation, mineralization and nutrient recycling. In this study, cellulase, xylanase and amylase were selected because they are very important in

carbon-cycle. β-glucosidase releases low molecular weight sugars, which are important as energy sources for soil micro-organisms. Urease and N-acetyl-glucosaminidase play an important role in nitrogen-cycle. Fluorescein diacetate (FDA) hydrolysis assay was used to measure enzyme activity of decomposer microorganisms, which are important in forest ecosystems for mineralization and turnover of organic matter. Both the soil microorganisms and macrofauna can modify the physical and chemical properties of the soil and can be used to monitor soil quality in agricultural land. The soil biochemical state has been suggested as sensitive indicator of soil ecological stress recovery processes in agro-ecosystems. Numerous studies have been published on the potential use of enzyme activities as indicators of soil productivity. Enzyme activities in soil have been used to investigate pesticide pollution (Sannino and Gianfreda, 2001), the impact of cultivation (Vepsäläinen *et al.,* 2001; Acosta-Martínez *et al.,* 2007) and metal pollution (Epelde *et al.,* 2008). Soil enzyme activities have also been proposed as indicators for suppressive defense against soil pathogens (Naseby *et al.,* 1998; Bruggen and Semenov, 2000; Rasmussen *et al.,* 2002; Leon *et al.,* 2006).

The relationship between plant and microorganisms are an important part of forest soil ecosystem. Soil microbes are dominant for soil quality because they are greatly important in soil functioning and fertility (Elliott *et al.,* 1996). Soil microbes can be used as sensitive indicator of disturbing soil ecology (Dick, 1992; Dilly and Blume, 1998). The microorganisms play role in soil nutrient cycling, because their extracellular enzymes have degraded organic matters as their energy source (Pavarina *et al.,* 1999; Vidal *et al.,* 1999; Tribak *et al.,* 2002; Khaid *et al.,* 2006). The capacity of microbes in decomposition depends on type and abundance of organic materials (Saliha *et al.,* 2005). Some microbes have ability to convert toxic chemical (such as cyanide) in soil to essential nutrient for plant (Maier-Greiner *et al.,* 1991). The microbes also influence soil physical property, especially, the stability of soil aggregation (Harris *et al.,* 1964; TonThat and Cochran, 2000), which benefit for water movement in soil and plant root growth. Soil microbial diversity is influenced by different factors including soil management system (Lucasa *et al.,* 2007), climate change (Freya *et al.,* 2008) and soil pollution (Kandeler *et al.,* 2000), and thus, soil microbial community structure has been noted as important for understanding the relationship between environmental factors and ecosystem functioning.

The effect of different factors on composition of soil microbial community can be investigated by both cultivation-dependent and cultivation-independent. However, the methods under cultivation-dependent still have limitation because numerous microorganisms in soil cannot culture in synthetic media under laboratory conditions (Borneman *et al.,* 1996;

Torsvik *et al.,* 1998; van Elsas *et al.,* 2000). To study these microbes, cultivation-independent methods have been invented for examination of environmental samples. The molecular biology techniques are powerful tools to detect the microbial populations present in soil both culturable and unculturable microorganisms. The denaturing gradient gel electrophoresis (DGGE) has been used widely for investigation of soil microbial community. This technique involves amplifying target-DNA region and PCR products with similar length are separated in polyacrylamide gel containing gradient of denaturing conditions (Kirk *et al.,* 2004; Nakatsu, 2007). The DGGE technique is reliable, reproducible and rapid to study soil microbial diversity (Duineveld *et al.,* 2001; Maarit-Niemi *et al.,* 2001). This technique is used for studying soil microbial community on agricultural practices (Nakatsu *et al.,* 2000; Hagn *et al.,* 2003; Siddique *et al.,* 2005; Sekiguchi *et al.,* 2008), their degrading substrates (van der Wal *et al.,* 2006; Cosgrove *et al.,* 2007; Das *et al.,* 2007) and plant genotypes (Ikeda *et al.,* 2006)

The biological indicators in soil under TPN affected tree merit study. The determination of macrofauna diversity and soil enzyme activities may be useful for obtaining a greater understanding of the impact of this disease on soil ecosystem functions. The main objectives of this study were (i) to investigate soil biological indicators in *H. brasiliensis* plantation, which had high incidence of TPN and (ii) to determine the differences in such indicators between the soils under trees affected by TPN and under healthy trees.

III.1.2 EXPERIMENTAL DESIGN

To determine the impact of TPN, TPN affected trees and healthy trees were selected using the annual tree health survey which carried out by D. Nandris. Ten plots were chosen and in each plot, samples were taken from four different sampling zones: near collar of affected tree (B), midway between affected trees and healthy trees (BH), near collar of healthy trees (H) and midway between healthy trees (HH) (Figure 2).

For enzyme assay and microbial culture, the soil samples were mixed using three identical sampling zones with depth. For genetically microbial diversity, the soil samples were mixed using three identical sampling zones with 0-5 cm and 5-10 cm depths.

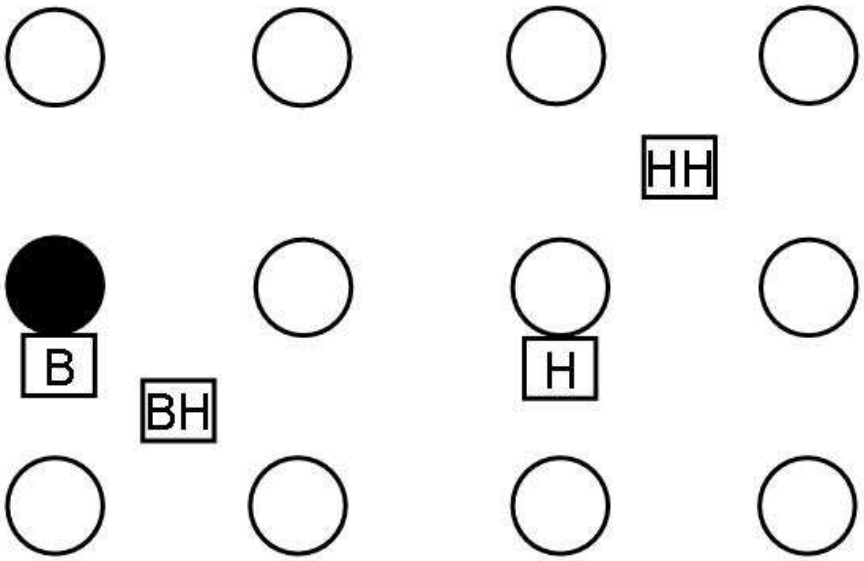

Figure 2 Sampling scheme in the rubber tree plantation: healthy tree (○), tree affected by TPN (●). B is near the collar of tree affected by TPN, BH is midway between affected tree and healthy tree, H is near the collar of a healthy tree, HH is midway between healthy rubber trees.

III.1.3 RELATIONSHIP BETWEEN TPN ANDDIFFERENT SOIL PROPERTIES

Soil properties in rainy and dry seasons were showed in table 2.

Soil pH

In rainy and dry seasons, no significant difference in soil pH was observed for soil under midway between healthy trees (HH), midway between TPN affected tree and healthy trees (BH), near collar of healthy tree (H) and near collar of TPN affected tree (B).

For each zone, soil are more acidic in rainy than in dry season

Organic carbon

In rainy season, organic carbon was the highest in soil under midway between healthy trees (HH) and was significantly higher ($P<0.05$) than under near collar of TPN affected tree (B).

In dry season, no significant difference was observed from whole samples.

Total nitrogen

No treatment was significantly different in soil nitrogen in rainy season.

For dry season, the soil in midway between healthy trees (HH) was significantly higher ($P<0.05$) than in near collar of trees (B and H).

C:N ratio

In rainy and dry seasons, no significant difference of the C:N ratio was observed between sampling zones.

Organic matter (OM)

The values of organic matter were the highest in soil under midway between healthy trees (HH) in both seasons. In rainy season, the OM content in soil was significantly lower ($P<0.05$) under near collar of TPN affected tree (B) than under midway between healthy trees (HH). No significant different in soil organic matter was observed in dry season.

Cation exchange capacity (CEC)

The evaluation of cation exchange capacity of soil in rainy and dry seasons showed that the CEC was significantly higher ($P<0.05$) in the soil under midway between TPN affected tree and healthy trees (BH) than under near collar of healthy tree (H).

Soil moisture

In rainy season, soil moisture was clearly higher than in dry season. For each season, the moisture under near collars of trees (B and H) was significantly lower ($P<0.05$) than under midway between trees (BH and HH).

Clay content

In rainy season, soil clay content was higher in healthy tree areas (H and HH) than in TPN areas (B and BH) and was the highest in midway between healthy trees (HH). However, no significant different was observed in both rainy and dry season.

Table 2 Soil properties in *H. brasiliensis* plantation under near collar of TPN affected tree (B), midway between TPN affected tree and healthy trees (BH), near collar of healthy tree (H) and midway between healthy trees (HH) in rainy and dry seasons.

	B	BH	H	HH
Rainy season				
pH	4.29 ± 0.30 a	4.58 ± 0.25 a	4.34 ± 0.34 a	4.50 ± 0.37 a
OC (%)	0.51 ± 0.11 a	0.76 ± 0.22 ab	0.55 ± 0.14 ab	0.78 ± 0.27 bc
Total N (%)	0.07 ± 0.01 a	0.09 ± 0.03 a	0.08 ± 0.02 a	0.08 ± 0.02 a
C:N ratio	7.02 ± 1.59 a	8.76 ± 2.00 a	7.09 ± 2.85 a	9.95 ± 3.20 a
OM (%)	0.88 ± 0.19 a	1.30 ± 0.39 ab	0.94 ± 0.24 ab	1.34 ± 0.48 b
CEC (%)	3.83 ± 0.26 a	5.21 ± 1.46 b	3.83 ± 0.39 a	4.43 ± 0.56 ab
Moisture (%)	11.63 ± 0.91 a	17.24 ± 2.21 b	10.62 ± 2.94 a	16.22 ± 2.30 b
Clay (%)	8.15 ± 2.26 a	8.43 ± 2.47 a	9.05 ± 1.79 a	9.54 ± 1.36 a
Dry season				
pH	5.02 + 0.26 a	5.08 + 0.54 a	5.06 + 0.32 a	5.02 + 0.37 a
OC (%)	0.53 + 0.06 a	0.57 + 0.28 a	0.52 + 0.06 a	0.71 + 0.20 a
Total N (%)	0.07 + 0.02 a	0.08 + 0.02 ab	0.06 + 0.01 a	0.11 + 0.03 b
C:N ratio	8.17 + 1.59 a	7.44 + 3.29 a	9.14 + 1.41 a	7.68 + 4.18 a
OM (%)	0.91 + 0.10 a	0.99 + 0.48 a	0.90 + 0.11 a	1.23 + 0.35 a
CEC (%)	4.13 + 0.52 ab	4.94 + 1.08 bc	3.85 + 0.48 a	5.20 + 0.86 c
Moisture (%)	1.71 + 0.93 a	3.18 + 0.60 b	1.57 + 0.98 a	3.99 + 0.65 b
Clay (%)	9.17 + 1.64 a	8.45 + 1.75 a	8.76 + 2.23 a	9.97 + 1.31 a

Means within the same column followed by contrasting letters are significantly different among treatment using TukeyHSD test ($P<0.05$) (±: standard deviation, n=9)

Multivariate analysis

Principal component analysis on soil variables with the results obtained in rainy and dry seasons were constructed (Figure 3). In rainy season, PCA showed axis 1 and axis 2 for 56.6% and 17.8% of the variance, respectively. In dry season, PCA showed axis 1 and axis 2 for 50.7% and 28.2% of the variance, respectively. Testing the significance of groupings (sites) was carried out on 10,000 permutations and was significantly discriminated ($P<0.00$) in rainy seasons. In both seasons, the PCA revealed that the soil under near collar of trees (B and H) was associated with the low values of soil variables. The high values of organic matter, organic carbon and CEC were associated with soil under midway between trees (BH and HH). The total N was associated with soil under midway between trees (BH and HH) in, particularly, dry season. The C:N ratio was associated with soil under midway between trees (BH and HH) in, particularly, rainy season.

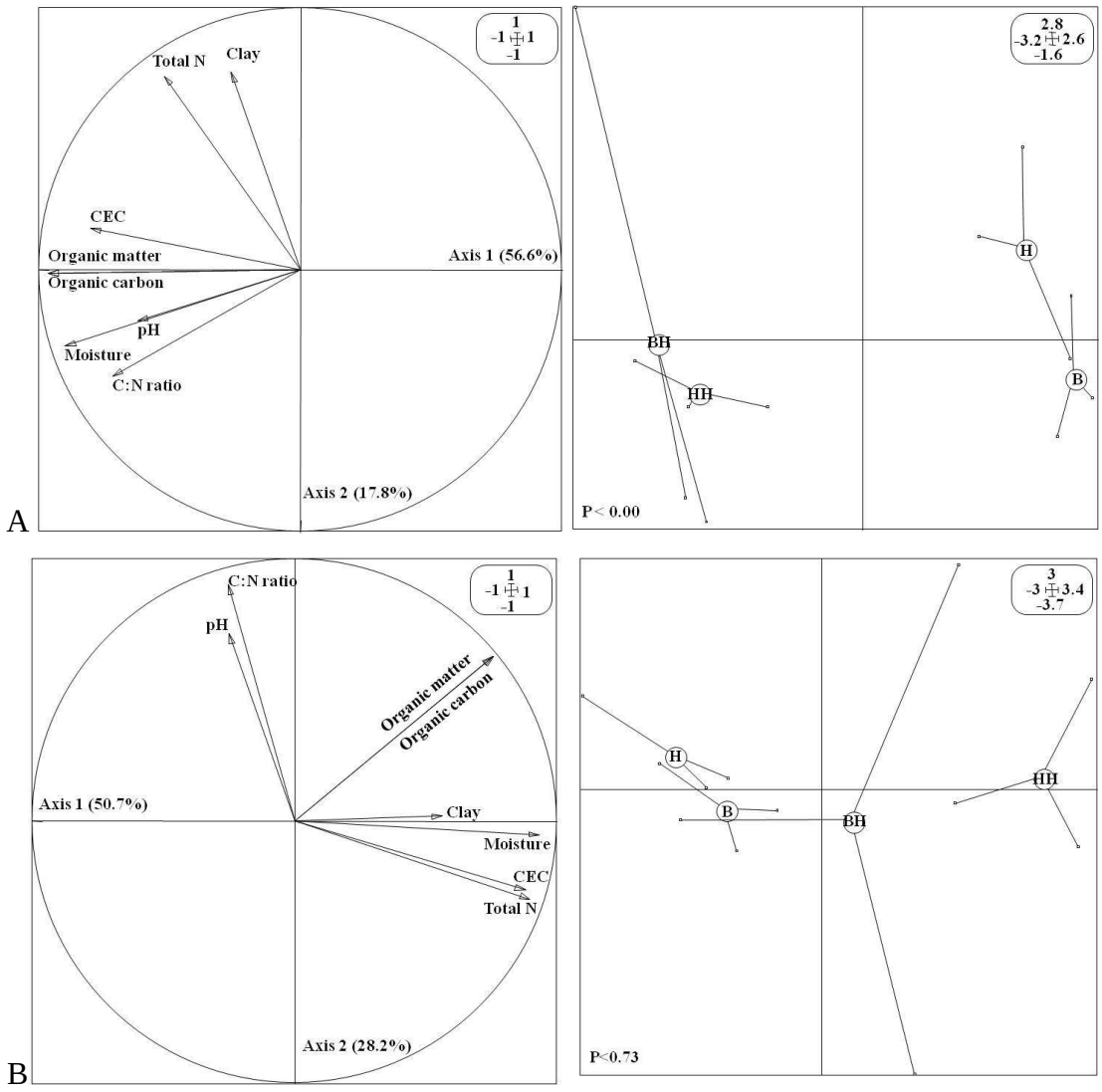

Figure 3 Principal component analysis showed the relationship between soil variable and sampling zones with near collar of TPN affected tree (B), near collar of healthy tree (H), midway between TPN affected tree and healthy tree (BH) and midway between healthy trees (HH) during raniy (A) and dry (B) season.

III.1.4 TPN AND SOIL MACOFAUNA

III.1.4.1 Macrofauna density

Litter macrofauna

The litter macrofauna was sampled in rainy and dry seasons including Coleoptera, termites, earthworms, Chilopoda, Diplopoda, Arachnida, ants and other invertebrate (Figure 4). In each season, the macrofauna was the most abundant in area under between tree affected by TPN and healthy trees (BH), followed by under between healthy trees (HH), near collar of TPN affected tree (B) and near collar of healthy tree (H) but densities were significantly different only in dry season.

During the rainy season, ants are the major group in the 4 sampling zones whereas "other group" was the dominate in the dry season. Densities of Arachnida and Coleoptera were quite the same in each zone and for each season. They were relatively stable groups. Termite density was the highest in HH zone during dry season. In rainy season, termites are only present near TPN zones (B, BH). Earthworm was collected only during rainy season between tree affected by TPN and healthy trees (BH). The density of "others" increased more than ten folds in dry season in comparison to rainy season. This important increase is due to an important density of Orthoptera, which does not present in rainy season.

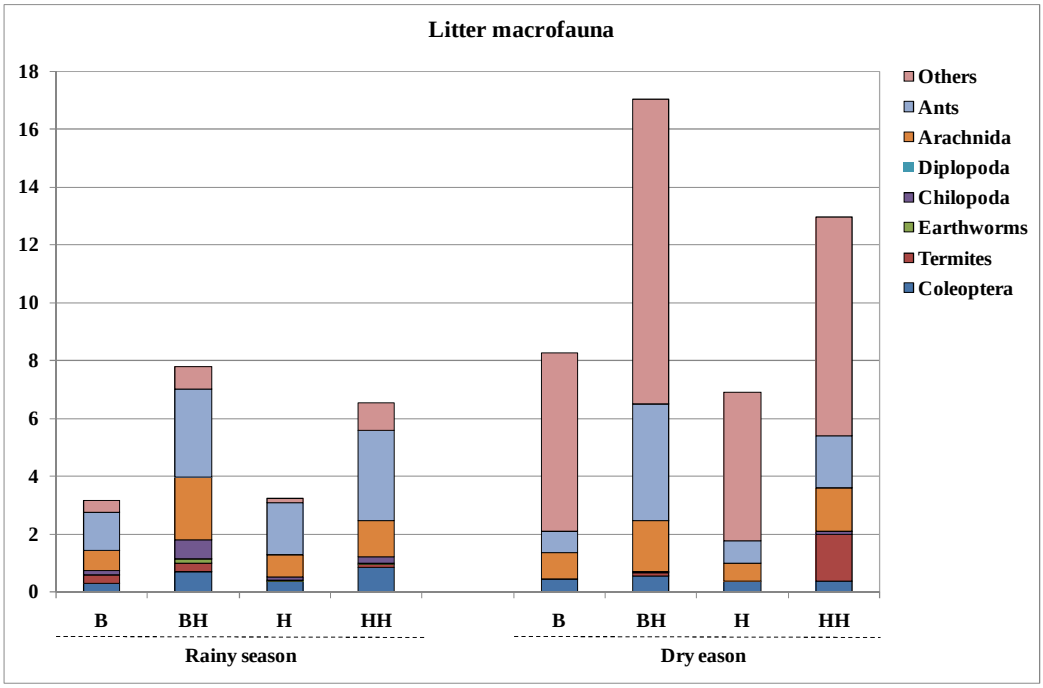

Figure 4 Density of macroinvertebrates in litter (individual m^{-2}) in the rainy and dry season

Soil macrofauna

There was great difference in the macrofauna density between rainy and dry season. In dry season, macrofauna was found only in TPN areas (B and BN). The density near collar of TPN tree (B) was significantly higher than between affected TPN and healthy trees (BH). Coleoptera and Chilopoda were only found under between TPN affected tree and healthy trees (BH). However, these invertebrates were present in low numbers. Furthermore, termite and ant were only found under near collar of TPN affected tree (B).

The soil macrofauna in rainy season included Coleoptera, termites, earthworms, Chilopoda, ants and other invertebrate (Figure 5). The macrofauna was the most abundant in soil near collar of TPN affected tree (B). The coleoptera density was the highest under between healthy trees (HH) while no Coleoptera was observed in area under near collar of TPN affected tree (B). Termite density was higher under TPN disease (B and BH) than under healthy tree (H and HH). The earthworm population was lower in areas near collr of trees (B and H) than areas midway between trees (BH and HH). The Chilopoda was only found under between healthy trees (HH). Abundance of ant was the highest in soil under between healthy trees (HH). In area near collar of TPN affected tree (B), only termite group was observed.

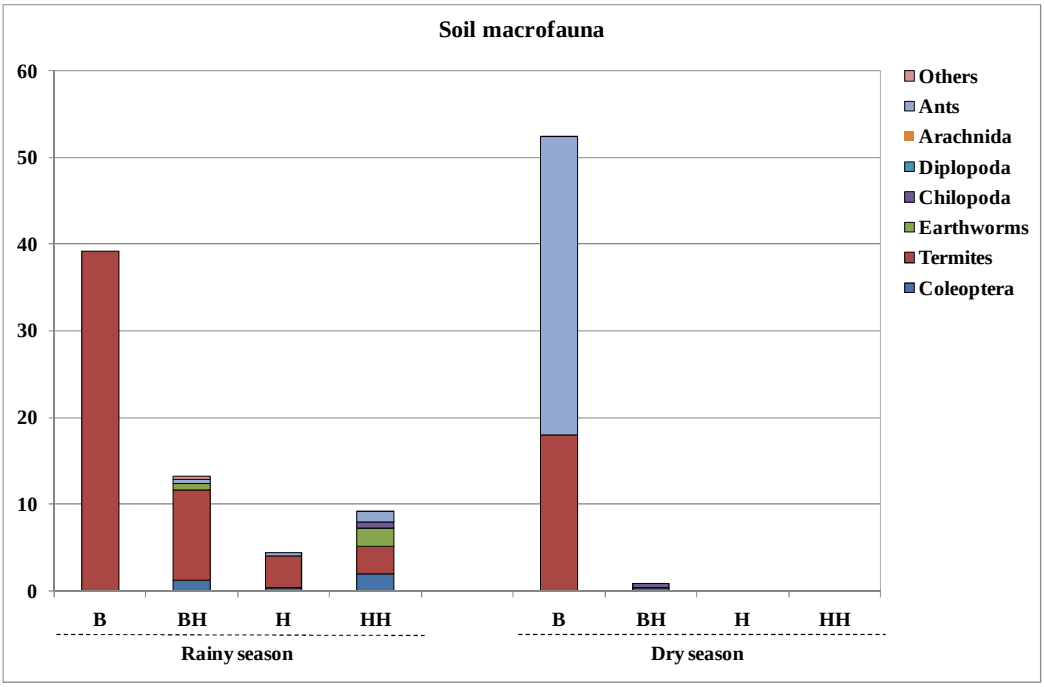

Figure 5 Density of macroinvertebrates in soil (individual m^{-2}) in the rainy and dry season

III.1.4.2 Macrofauna diversity

Diversities of litter and soil macrofauna were showed in table 3.

Litter macrofauna

In dry and rainy seasons, the diversity of Coleoptera was the highest under between healthy trees (HH) (included Carabidae, Elateridae, Scarabaeidae, Staphylinidae, Tenebrionidae, Dysticidae and larva). In rainy season, the termite diversity was the highest under near collar of TPN affected tree (B) (included two species collected *Ancistrotermes* sp. and *Macrotermes* sp.) while *Macrotermes* sp. was only found in litter under BH and HH zones. In contrary, in dry season, *Ancistrotermes* sp. and *Macrotermes* sp. were found in all sampling zones.

For the two sampling period, ant diversity was the highest under between healthy trees (HH) but the species richness of ant was the highest in rainy season.

Soil macrofauna

In rainy season, the diversity of Coleoptera was the highest under between healthy trees (HH) (included Scarabaeidae, Tenebrionidae and larva) while soil under between TPN affected tree and healthy trees (BH), and near collar of healthy tree (H) have only one Coleoptera species (included larva and Carabidae, respectively). The termite *Ancistrotermes* sp. and *Macrotermes* sp. were found in all sampling zones. Ant diversity was the highest under between healthy trees (HH) (*Morphospecies* sp. 2 and larva) while ant *Camponotus* sp. 3 and *Odontoponera transvers* were observed under between TPN affected tree and healthy trees (BH), and near collar of healthy tree (H), respectively.

In dry season, Coleoptera Tenebrionidae and Chilopoda were only found under between TPN affected tree and healthy trees (BH). However, these invertebrates were present in low numbers. Furthermore, termite (*Macrotermes* sp.) and ant (*Calyptomyrmex* sp.) were only found under near collar of TPN affected tree (B).

Table 3 Diversity of macrofauna in soil and litter under near collar of affected tree (B), midway between affected trees and healthy trees (BH), near collar of healthy trees (H) and midway between healthy trees (HH) during rainy and dry seasons

	Litter								**Soil**							
	Rainy				**Dry**				**Rainy**				**Dry**			
	B	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**
Coleoptera																
Carabidae sp.	*	*		*	*	*		*			*					
Cerambycidae sp.			*		*		*									
Coccinellidae sp.																
Elateridae sp.				*	*											
Scarabaeidae sp.				*	*		*					*				
Staphylinidae sp.	*			*		*		*								
Tenebrionidae sp.	*	*	*	*	*	*	*	*				*		*		
Dytiscidae sp.				*												
Larva	*			*	*	*				*		*				
Termites																
Ancistrotermes sp.	*					*			*	*	*	*				
Macrotermes sp.	*	*		*		*		*	*	*	*	*	*			
Ants																
Odontoponera transversa	*	*	*	*							*					
Pachycondyla sp.					*	*		*								
Platythyrea sp.						*		*								
Pheidole sp. 1																
Pheidole sp. 2			*													
Morphospecies sp. 2												*				
Myrmecina sp.					*											
Calyptomyrmex sp.													*			
Paratrechina longicornis	*		*	*												
Anoploepis gracilipes				*	*		*									
Oecophylla longinoda	*	*	*	*												
Camponotus sp. 1	*	*		*	*	*										
Camponotus sp. 2	*	*	*		*	*	*	*								

	Litter								Soil							
	Rainy				Dry				Rainy				Dry			
	B	BH	H	HH	B	BH	H	HH	B	BH	H	HH	B	BH	H	HH
Camponotus sp. 3	*	*	*	*		*		*		*						
Camponotus sp. 4		*														
Camponotus sp. 5	*	*	*	*												
Camponotus sp. 6			*													
Larva						*		*				*				

(Table 3 continue)

Diversity index

The specific data obtained on the major group were used to calculate several diversity indexes: specific richness, Shannon index and diversity Simpson index (Table 4).

The specific richness considered that only the number of species or morphospecies was higher under near collar of TPN tree (B) and between healthy trees (HH). However, the analysis of the Shannon and Simpson diversity indexes showed that these two zones were clearly different. The zone under midway between healthy trees (HH) was high in both indexes (1.10 and 0.90, respectively), this indicated that the macrofauna community was composed by species with equivalent population.. For the zone under near collar of TPN tree (B), both indexes were low (0.54 and 0.67, respectively). This demonstrated that there are few species dominated by a great number of individuals whereas other species were represented by few individuals. Thus, the macrofauna community was equilibrated in area of midway between healthy trees (HH) whereas it was incompletely equilibrated in area of near collar of TPN tree (B). Thus, the macrofauna community was equilibrated in area of midway between healthy trees (HH) whereas it was incompletely equilibrated in area of near collar of TPN tree (B).

On the other hand, the similarity index of Jacquard was constructed (Table 5) and showed that the macrofauna composition in condition of identical sampling zones (H and B; 0.79, and BH and HH; 0.80) was higher similar than in different sampling zones (H and HH; 0.71, and BH and H; 0.62). This indicated that the influence of conditions of sampling zones were important more than the presence of TPN disease.

Table 4 Specific richness, Shannon index and Simpson index of macrofauna

	Specific richness	**Shannon**	**Simpson**
B	20	0.54c	0.67c
BH	16	0.93b	0.83b
H	15	0.90b	0.83b
HH	21	1.10a	0.90a

Table 5 Jacquard similarity index of macrofauna

	B	BH	H	HH
B	1	0.77	0.79	0.78
BH		1	0.62	0.80
H			1	0.71
HH				1

Conclusion

The macrofauna diversity has used as biological indicator of soil disturbance because it reflects the impact of environmental change (Nahmani *et al.,* 2006; Velasquez *et al.,* 2007; Yankelevich *et al.,* 2007). In rainy season, most invertebrates were associated with areas between trees (BH and HH) more than areas near collars of trees (B and H). The areas between trees in this plantation were accumulated with organic materials, especially, litter. The higher food source influences macrofaunal population and diversity. In rainy season, the difference in Coleoptera abundance between BH and HH was not obviously different but the diversity was the highest in area between healthy trees (HH). Likewise, the earthworm population was mostly found in soil under between healthy trees (HH). For the tropical forest, the quality of litter has been showed to affect macrofaunal population (Zou, 1993; Mboukou-Kimbatsa *et al.,* 1998; Gonzalez and Zou, 1999). The quality of plant materials depends on chemical component, which is the important factor to regulate the macrofaunal abundance and diversity. As mentioned above, the TPN affected tree may contain tannins more than healthy tree. The exceeding tannins can adversely affect some invertebrates. Tannins are binding protein agents, which are able to bind digestive enzymes to limit assimilation in insects. This has effect on survival and growth of macrofauna. Consequently, macrofaunal abundance and diversity was low (Loranger-Merciris *et al.,* 2007). However, (Ayres *et al.,* 1997) suggested that the same tannin had different effect on different herbivore insects. In this plantation, Coleoptera genus *Cerambycidae*, *Elateridae*, *Scarabaeidae* and *Dytiscidae* were not found in both soil and litter under TPN affected trees (B and BH). These Coleoptera genera may be affected by tannins from trees affected by TPN. On the other hand, some insect species in Carabidae and Tenebrionidae were detected in all sampling zones. These macrofaunas may resist polyphenols.

The termite density was higher under TPN affected trees (B and BH). Severe TPN causes the bark to slough off at the collar. As only healthy bark containing latex and cyanide is able to block boring insects, the unprotected wood can be attacked by termites to become part of their habitat. Consequently, the zones with the highest termite population were strongly associated with areas under the affected trees.

In dry season, soil macrofaunal diversity in dry season was lower than in rainy season. The Coleoptera was detected only in soil under between TPN affected tree and health trees (BH) but its diversity was very low, only Tenebrionidae was found. This may be due to climate condition. The climate is considered to be the most important factor in the regulation of macrofaunal community. In drought circumstance, soil was arid and hard because of

decreasing soil water levels. Soil moisture, which is greatly associated with seasonal variation, significantly affects soil macrofauna (Sroka and Finch, 2006). In this season, no earthworm was found in all sampling zones because the earthworms maybe migrate to deeper soil layers. In during winter and summer, the earthworms can reach a depth of 40-45 cm and was in quiescent stage (Reddy and Pasha, 1993) whereas macrofauna was collected in a maximum depth of 30 cm in this study.

III.1.5 TPN AND SOIL ENZYME ACTIVITY

III.1.5.1 Rainy season

These results have been published in Forest Pathology

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Biological activity of soils under rubber trees (*Hevea brasiliensis*) affected by trunk phloem necrosis

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Summary

Trunk phloem necrosis (TPN) is currently one of the most economically important diseases of the rubber tree (*Hevea brasiliensis*). Investigations of the aetiology of the disorder have failed to identify any biotic causal agents but a multidisciplinary study has recently suggested that the disease may be caused by a combination of exogenous and endogenous stresses. These stresses can affect plant physiology and may also have an impact on soil biology and soil biochemistry. In this study, macrofauna diversity and soil enzyme activities were compared for the soils under trees affected by TPN and under healthy trees. Principal component analysis revealed associations between TPN and macrofauna diversity and soil enzyme activities. Groups of ants and termites were associated with the soil under healthy and trees affected by TPN respectively. Polysaccharidase (cellulase, xylanase and, particularly, amylase) activities were lower in the soil under trees affected by TPN whereas *N*-acetylglucosaminidase activity was higher. These findings suggested that termite density and *N*-acetylglucosaminidase activity are useful indicators of TPN, while lower values of polysaccharidases activities are the consequence of this disease. The impact of TPN on soil ecosystem was discussed.

1 Introduction

The rubber tree (*Hevea brasiliensis*) is an important source of natural rubber. Rubber tree bark necrosis [Trunk phloem necrosis (TPN)] has become a serious problem in rubber tree plantations because this disease prevents latex production. TPN was first described in the 1980s in Ivory Coast by Nandris et al. (1991a). The first visible symptoms are necrosis of the bark at the collar with thin cracks that extend upward towards the tapping panel. When the inner phloem, which contains the laticifers, becomes affected, the latex production is affected and the 'panel cut' becomes dry (Nicole et al. 1991). On-going and completed studies to identify a pathogen have failed to confirm any causal agents such as fungi and fungus-like organisms (e.g. *Phytophthora*, *Pythium*, *Fusarium*), bacteria, phytoplasma, viruses or viroids (Nandris et al. 1991b; Pellegrin et al. 2007). In addition, several experiments (bud grafting, bark implantation, tapping knife disinfection) and the spatio-temporal modelling of TPN dynamics have shown no evidence of pathogen transmission (Peyrard et al. 2006).

The first breakthrough on TPN was reported by Nandris et al. (2004, 2005, 2006) who concluded that this disease may be caused by a combination of exogenous and endogenous

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stresses. In particular, soil compaction which can induce water stress affecting the health of the trees and low soil fertility were thought to increase the risk of the syndrome. Furthermore, the non-optimal vascular interface between the rootstock and the scion, combined with a major physiological dysfunction (cell disruption) can lead to the release of native cyanide in the inner bark, thus causing cell death and spread of necrosis in the phloem (CHERSTIN et al. 2004). All these stresses may disrupt physiological metabolisms of rubber trees and consequently affect the soil biological microenvironment around the tree. However, the real impact of TPN on soil ecology is unknown.

Soil is a basic resource for agricultural production systems. Soil quality is fundamental to the success of crop production systems and is associated with soil biological activity. Soil macrofauna, considered to be ecosystem engineers (*sensu* JONES et al. 1994), play an important role in soil formation, affecting organic matter decomposition and nutrient cycling. Increasing soil macrofauna diversity tends to increase the density of soil microorganisms as macrofauna can modify the conditions for microorganisms which are important sources of enzymes in soil (MORA et al. 2005). Enzymes are the main mediators of soil biological processes: organic matter degradation, mineralization and nutrient cycling. Both the soil microorganisms and macrofauna can modify the physical and chemical properties of the soil and can be used to monitor soil quality in agricultural land. The soil biochemical state has been suggested as sensitive indicator of soil ecological stress recovery processes in agro-ecosystems. Numerous studies have been published on the potential use of enzyme activities as indicators of soil productivity. For example, enzyme activities in soil have been used to investigate pesticide pollution (SANNINO and GIANFREDA 2001), the impact of cultivation (VEPSÄLÄINEN et al. 2001; ACOSTA-MARTÍNEZ et al. 2007) and metal pollution (EPELDE et al. 2008, Li et al. 2009). Soil enzyme activities have also been proposed as indicators for suppressive defence against soil pathogens (NASEBY et al. 1998; BRUGGEN and SEMENOV 2000; RASMUSSEN et al. 2002; LEON et al. 2006).

Biological indicators in soil under rubber trees affected by TPN merit study. The determination of macrofauna diversity and soil enzyme activities may be useful for obtaining a greater understanding of the relationship between this disease and soil ecosystem functions. The main objectives of this study were (i) to investigate soil biological indicators in a rubber tree plantation which had high incidence of TPN and (ii) to determine the differences in such indicators between the soils under trees affected by TPN and under healthy trees.

2 Materials and methods

2.1 Field site description

The study was conducted in the 9-year-old rubber plantation in Buri Rum province, northeastern Thailand (15°16′18′N and 103°0′6′E). The climate is tropical with the rainy season occurring from May to October. During the 5-year period 2001–2006, the mean annual rainfall was 1207 mm (which is very low for rubber plantations) and the mean annual temperature was 26°C. The soil is sandy loam with 67, 24 and 9% of sand, silt and clay, respectively. The rubber tree plantation was characterized by low input farming and a high incidence of TPN (20–25% of trees affected). The study site was sampled during the rainy season (July to October 2006).

2.2 Experimental design

Trees affected by TPN and healthy trees were selected using the annual tree health survey carried out by D. Nandris. Ten plots were chosen and in each plot, four different

sampling zones were selected: next to affected trees (B), half-way between affected trees and healthy trees (BH), next to healthy trees (H) and half-way between healthy trees (HH) (Fig. 1).

2.3 Sampling design

2.3.1 Sampling of macrofauna

The tropical soil biology and fertility method was used to sample soil macrofauna (Anderson and Ingram 1993). A block of soil (25 × 25 × 30 cm) was taken and macrofauna collected at three depths (0–10, 10–20 and 20–30 cm). The macrofauna were hand sorted to extract invertebrates with a body length greater than 2 mm. Invertebrates were preserved in 4% formalin and separated into nine broad taxa (orders or families).

2.3.2 Sampling of soil and preparation

For sampling the soils in each zone, a block (12 × 12 × 10 cm) was taken using hand trowel. The block was separated into three layers (0–2, 2–5 and 5–10 cm). The samples were air-dried, ground and sieved to 2 mm. Plant residues and roots were removed. Three identical sampling zones with depth, samples were mixed for enzyme activity analysis.

2.4 Soil enzyme activity

To evaluate soil enzyme activity, the soil samples were incubated with purified water (1 g/150 μl) at 25°C for 48 h to reactivate the microflora. Enzyme assays were carried out in triplicate to measure activities. β-Glucosidase, *N*-acetyl-glucosaminidase, amylase, cellulase and xylanase activities were assayed using a modification of the procedure described by Mora et al. (2005). The modified Berthelot reaction (Kandeler et al. 1999) was used to measure urease (EC 3.5.1.5) activity. Total microbial activity potential was measured using fluorescein diacetate (FDA) hydrolysis in which colourless FDA is hydrolysed releasing the coloured end product fluorescein (Green et al. 2006).

β-Glucosidase and *N*-acetyl-glucosaminidase activities were assayed using *p*-nitrophenyl β-D-glucopyranoside and *p*-nitrophenyl *N*-acetyl glucosaminide (Sigma, St. Louis, MO, USA) as substrates. Soil (200 mg) was incubated at 37°C for 2 h with 200 μl of 1% (w/v) substrate and 100 μl of citrate phosphate buffer at pH 5.8. The reaction was stopped with 2% Na_2CO_3 (w/v). After centrifugation at 12000 ***g*** for 5 min, absorbance was measured at 420 nm. Enzyme activities were expressed as μg PNP released g^{-1} soil h^{-1}.

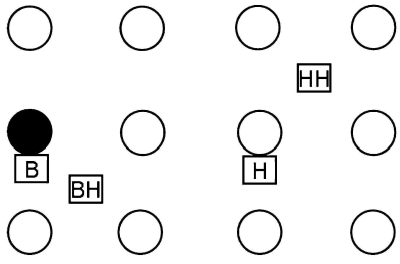

Fig. 1. Sampling scheme in the rubber tree plantation: healthy tree (○), tree affected by TPN (●). B is near the collar of tree affected by TPN, BH is midway between affected tree and healthy tree, H is near the collar of a healthy tree, HH is midway between healthy rubber trees.

Activities of amylase, cellulase and xylanase were determined using starch, carboxymethyl-cellulose and xylan as substrates, respectively. Soil (200 mg) was incubated with 200 μl of 1% (w/v) substrate and 100 μl of citrate phosphate buffer at pH 5.8 at 37°C for 4 h. After incubation, reducing sugars were measured using the method of SOMOGYI (1945) and NELSON (1944). Absorbance was measured at 650 nm and the results were expressed as μg glucose released g^{-1} soil h^{-1}.

For urease activity, 200 mg of soil was mixed with 400 μl of 100 mM borate buffer (pH 10.0) and 50 μl of 720 mM urea. After incubation at 37°C for 4 h, the reaction was stopped with 3 ml acidified 2 M KCl and 1.5 ml of the mixture was centrifuged at 13000 rpm for 5 min. One millilitre of the supernatant was mixed with 5 ml of a 1 : 1 : 1 (v/v/v) mixture of sodium salicylate/sodium nitroprusiate reagent (17%, w/v and 0.12%, w/v, respectively), 0.3 M NaOH and distilled water. Finally, 2 ml of 39.1 mM of sodium dichloroisocyanurate and 2 ml of distilled water were added. After agitation in the dark for 30 min, absorbance was measured at 660 nm and enzyme activity expressed as μg $N\text{-}NH_4^+$ g^{-1} soil h^{-1}.

For FDA hydrolysis, 200 mg of soil was incubated for 2 h at 37°C, with 25 μl of 4.8 mM FDA solution and 1.5 ml of a citrate phosphate buffer at pH 5.8. After incubation, the suspension was centrifuged at 13000 rpm at room temperature for 5 min and 750 μl of the supernatant was taken. The activity was stopped by adding 750 μl of acetone. The amount of FDA hydrolysed was measured as absorbance at 490 nm and the activities were expressed as μg fluorescein g^{-1} h^{-1}.

2.5 Statistical analysis

The relationship between macrofauna diversity and enzyme activities and the different sampling zones was determined by principal component analysis (PCA) using ADE-4 software (THIOULOUSE et al. 1997). A permutation test was used to check the significance of the groups suggested by PCA. ANOVA was used to apply the Tukey test ($p < 0.05$).

3 Results

3.1 Macrofauna

The soil macrofauna density was higher near the collars of the affected trees (B); it was not significantly different in between the trees (BH and HH). Near the collars of the healthy trees (H), the macrofauna density was clearly lower than in the other zones (Fig. 2).

Termites were the largest taxonomic group near the collars of the trees and were the only macrofauna group near the affected trees (B) and in between healthy and affected trees (BH). The macrofauna diversity was higher in both zones between trees than near the collars of the trees (Fig. 3). Although the macrofauna density was low between healthy trees (HH), the diversity was the highest.

3.2 Enzyme activities

3.2.1 Polysaccharidases

Cellulase, xylanase and amylase activities were lowest in soil sampled near the collars of affected trees (B) and lower between affected and health trees (BH) than near healthy trees (H and HH) (Table 1). Xylanase and amylase activities near the collars of the affected trees (B) were significantly lower ($p < 0.05$) than near healthy trees. The soil under healthy trees had the highest amylase activity, significantly higher than under affected trees (Table 1).

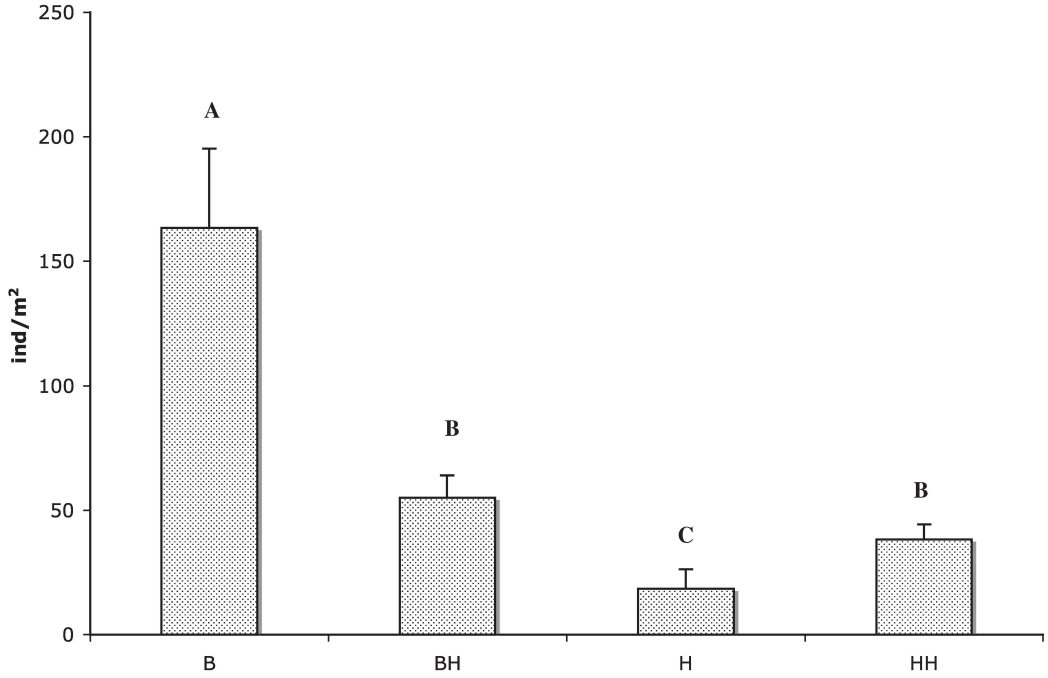

Fig. 2. Density of soil macrofauna in the four zones (B, BH, H and HH).

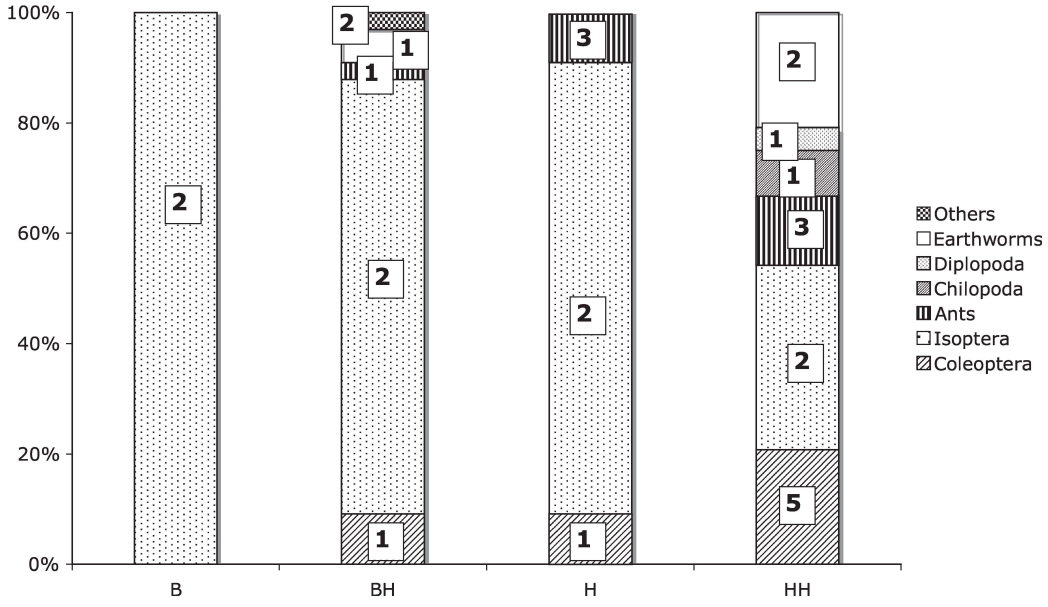

Fig. 3. Macrofauna diversity: proportions of each taxonomic group in sampling zones, number of species in the taxon.

3.2.2 Heterosidases

β-Glucosidase, *N*-acetyl-glucosaminidase hydrolysis activities were not significantly different between soil sampled near affected trees or healthy trees.

3.2.3 Urease

The highest values of urease were between healthy and affected trees (BH) and were significantly higher than for the soil close to both affected and healthy trees (B and H). There was no significant difference in activity for the samples taken from between trees (BH and HH) (Table 1).

3.2.4 FDA hydrolysis

Fluorescein diacetate hydrolysis activities of the soil samples collected in the various zones were not significantly different (Table 1).

3.3 Multivariate analysis

Principal component analysis showed that the soil under healthy trees (H) was associated with the highest amylase activity and the highest density of ants along axis 1 (Fig. 4). For the soil under affected trees (B), all the enzyme activities tested were low while the termite density was the highest. Soil samples with high cellulase, xylanase and amylase activities were associated with healthy trees (H and HH). Multivariate analysis revealed that the highest *N*-acetyl-glucosaminidase activity was associated with the soil between affected and healthy trees (BH). *β*-Glucosidase, urease and FDA hydrolysis activities were higher in sampling areas between trees (HH and BH).

4 Discussion

Macrofauna diversity was used as a biological indicator of soil disturbance because it reflects the impact of ecological stresses (Velasquez et al. 2007). Termite density was highest near affected trees (B and BH). Severe TPN causes the bark to slough off at the collar. As only healthy bark containing latex and cyanide is able to block boring insects, the unprotected wood can be attacked by termites to become part of their habitat. Consequently, zones with the highest termite populations were strongly associated with the soil under the affected trees.

Principal component analysis revealed that most of the enzyme activities were low near the collars of the rubber trees (B and H), as these zones did not accumulate leaf litter that is a major source of soil organic matter. Plant litter is an importance source of nutrients for soil microorganisms (Bardgett and Shine 1999; Ruan et al. 2005; Abraham and Chudek 2008) and litter input can increase soil enzyme activity (Dornbush 2007). High urease, *β*-glucosidase and FDA hydrolysis activities were found between the trees (HH and BH

Table 1. **Soil enzyme activities (μg of product g^{-1} soil h^{-1}) in four different zones; near collar of tree affected by TPN (B), midway between tree affected by TPN and healthy tree (BH), near collar of healthy tree (H) and midway between healthy trees (HH).**

	Cellulase	Xylanase	Amylase	*β*-Glucosidase	*N*-Acetyl-glucosaminidase	Urease	FDA hydrolysis
B	0.3 ± 0.5 a	0.8 ± 1.6 a	2.3 ± 2.2 a	11.1 ± 8.2 a	1.1 ± 0.8 a	0.8 ± 0.3 ab	3.1 ± 1.0 a
BH	1.0 ± 0.8 ac	2.8 ± 0.8 b	6.7 ± 1.6 a	29.8 ± 28.6 a	1.2 ± 0.7 a	2.0 ± 0.9 c	4.7 ± 2.6 a
H	0.7 ± 0.7 ac	3.2 ± 1.2 b	22.9 ± 7.8 c	8.6 ± 5.8 a	0.9 ± 0.8 a	0.7 ± 0.5 a	3.4 ± 1.0 a
HH	1.5 ± 1.0 bc	3.8 ± 1.1 b	12.9 ± 2.9 b	32.2 ± 32.6 a	1.3 ± 0.6 a	1.7 ± 1.1 bc	5.3 ± 2.3 a

Means within the same row followed by different letters are significantly different using Tukey HSD test ($p < 0.05$) (±: standard deviation, n = 9).

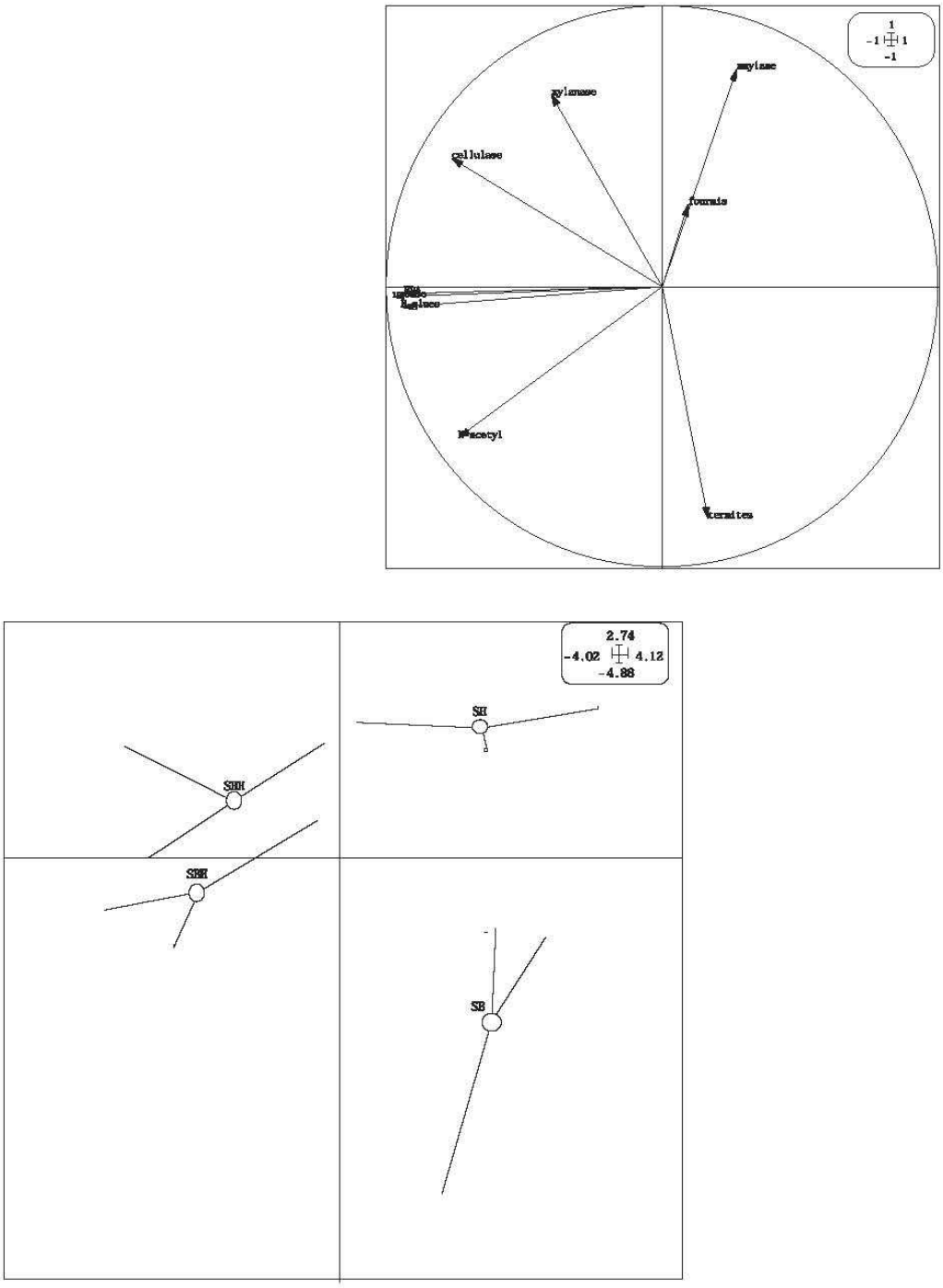

Fig. 4. Principal component analysis in four different zones.

zone). These zones accumulated abundant litter and thus the environmental conditions (such as soil microorganisms, humidity, etc.) producing these enzymes were similar.

Cellulase, xylanase and amylase activities were higher near healthy rubber trees (H and HH) than affected trees (B and BH) (Fig. 2). Thus, healthy trees were strongly associated

with polysaccharidases which can break down carbohydrates and release glucose as a source of available energy. These enzyme activities were rather low near affected trees. This effect may result from the difference in chemical composition between the leaves from healthy and affected trees which could have an impact on the quality of the litter and consequently on enzyme activities. Numerous studies have shown that litter quality influences various enzyme activities, increasing or decreasing the litter decomposition in the soil (Fioretto et al. 2000; Kourtev et al. 2002; Güsewell and Freeman 2005). In the rubber tree plantation in this study, dead plant materials or plant components (such as bark, branches, litter and latex) were the main source of organic matter for decomposition. The accumulation of defensive chemicals in the leaves or plant components of affected trees may affect soil enzyme activities. Some papers have reported that TPN may be caused by multiple exogenous and endogenous stresses (Nandris et al. 2004, 2005, 2006). Tapping bark for latex (too frequently, badly carried out or using chemicals for latex stimulation) contributes to the cumulative stress. Rubber trees can respond to such wounds or stresses by producing defensive chemicals including polyphenol as tannins (Eyles et al. 2004). Tannins can inhibit the activity of extracellular microbial enzymes (Scalbert 1991) including polysaccharidases such as cellulase and xylanase (Gamble et al. 2000; Barahona et al. 2006). Furthermore, high levels of tannins may influence soil mineralization and humus formation, decreasing soil fertility (Bradley et al. 2000; Driebe and Whitham 2000; Kraus et al. 2004; Nierop et al. 2006; Wurzburger and Hendrick 2007).

Principal component analysis showed that *N*-acetyl-glucosaminidase activity was associated with the area between health and affected trees (BH). *N*-Acetyl-glucosaminidase is one of the enzymes involved in chitin degradation (Ekenler and Tabatabai 2002). Chitinase has been found in plant tissue such as seed, bark, stems, leaves and roots (Hernes and Hedges 2004; Kosola et al. 2004; Kraus et al. 2004; Santos et al. 2004) but are also produced by microorganisms. In plant, this enzyme is rarely detectable in healthy plants but can be produced in reaction to damage or stress caused by various biotic or abiotic factors (Mauch et al. 1988). *Hevea brasiliensis* can produce chitinases (Bokma et al. 2002) with antifungal activity against several pathogenic fungi (Parijs et al. 1991; Giordani et al. 2002; Kanokwiroon et al. 2008). Chitinase activity is high in laticifers producing latex but low in healthy tissues such as leaves, stems or roots (Martin 1991). However, it would be important to determine the origin (trees or microorganisms) of *N*-acetyl-glucosaminidase activity detected in the BH to understand why it is produced only in this zone.

This study showed that TPN of *H. brasiliensis* has strong long-term effects on the soil ecosystem and soil fertility. PCA showed that the soil near affected rubber trees has lower polysaccharidase activities. This may be the result of secondary metabolites produced by affected trees. As little is known about the relationship between TPN and defensive chemicals and the impact of these agents on soil enzymes in rubber plantations, research is required into the secondary metabolites of trees affected by TPN.

5 Conclusion

The PCA carried out in this study showed for the first time that some biological activity indicators can help to distinguish between healthy trees (H and HH) and trees affected by TPN (B and BH). This distinction seems to be useful for observing the impact of TPN on the ecology of rubber tree plantations. Termite density was strongly associated with TPN (B and BH). High cellulase, xylanase and amylase activities were associated with healthy trees and were lower near diseased trees. PCA also indicated that *N*-acetyl-glucosaminidase activity was associated with the zone between healthy and affected trees (BH). It is suggested, therefore, that termite density, polysaccharidase activities and

N-acetyl-glucosaminidase activity can be used as indicators of the impact of TPN on soil ecology. However, the production of secondary metabolites as a result of TPN must be investigated. Further research needs to be carried out to assess the biological activities during the dry season in order to confirm this hypothesis.

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References

Abraham, J.; Chudek, J. A., 2008: Studies on litter characterization using ^{13}C NMR and assessment of microbial activity in natural forest and plantation crops' (teak and rubber) soil ecosystems of Kerala, India. Plant Soil **303,** 265–273.

Acosta-Martínez, V.; Cruz, L.; Sotomayor-Ramírez, D.; Pérez-Alegría, L., 2007: Enzyme activities as a affected by soil properties and land use in a tropical watershed. Appl. Soil Ecol. **35,** 35–45.

Anderson, J. D.; Ingram, J. S. I. 1993: Tropical Soil Biology and Fertility: A Handbook of Methods. 2. Wallingford: CAB International, pp. 171.

Barahona, R.; Sanchez, S.; Lascano, C. E.; Owen, E.; Morris, P.; Theodorou, M. K., 2006: Effect of condensed tannins from tropical legumes on the activity of fibrolytic enzymes from the rumen fungus *Neocallimastyx hurleyensis*. Enzyme Microb. Technol. **39,** 281–288.

Bardgett, R. D.; Shine, A., 1999: Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol. Biochem. **31,** 317–321.

Bokma, E.; Rozeboom, H. T. J.; Sibbald, M.; Dijkstra, B. W.; Beintema, J. J., 2002: Expression and characterization of active site mutants of hevamine, a chitinase from the rubber tree Hevea brasiliensis. Eur. J. Biochem. **269,** 893–901.

Bradley, R. L.; Titus, B. D.; Preston, C. P., 2000: Changes to mineral N cycling and microbial communities in black spruce humus after additions of $(NH_4)_2SO_4$ and condensed tannins extracted from *Kalmia angustifolia* and balsam fir. Soil Biol. Biochem. **32,** 1227–1240.

Bruggen, A. H. C. V.; Semenov, A. M., 2000: In search of biological indicators for soil health and disease suppression. Appl. Soil Ecol. **15,** 13–24.

Cherstin, H.; Sookmark, U.; Trouslot, P.; Pellegrin, F.; Nandris, D., 2004: Rubber tree (*Heave brasiliensis*) bark necrosis syndrome III: a physiological disease linked to impaired cyanide metabolism. Plant Dis. **88,** 1047.

Dornbush, M. E., 2007: Grasses, litter and their interaction affect microbial biomass and soil enzyme activity. Soil Biol. Biochem. **39,** 2241–2249.

Driebe, E. M.; Whitham, T. G., 2000: Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia **123,** 99–107.

Ekenler, M.; Tabatabai, M. A., 2002: Effects of trace elements on β-glucosaminidase activity in soils. Soil Biol. Biochem. **34,** 1829–1832.

Epelde, L.; Becerril, J. M.; Hernandez-Allica, J.; Barrutia., O.; Garbisu, C., 2008: Functional diversity as indicator of the recovery of soil health derived from *Thlaspi caerulescens* growth and metal phytoextraction. Appl. Soil Ecol. **39,** 299–310.

Eyles, A.; Davies, N. W.; Mitsunaga, T.; Mihara, R.; Mohammed, C., 2004: Role of *Eucalyptus globulus* wound wood extractives: evidence of superoxide dismutase-like activity. For. Pathol. **34,** 225–232.

Fioretto, A.; Papa, S.; Curcio, E.; Sorrentino, G.; Fuggi, A., 2000: Enzyme dynamics on decomposing leaf litter of *Cistus incanus* and *Myrtus communis* in a Mediterranean ecosystem. Soil Biol. Biochem. **32,** 1847–1855.

Gamble, G. R.; Snook, M. E.; Henriksson, G.; Akin, D. E., 2000: Phenolic constituents in flax bast tissue and inhibition of cellulase and pectinase. Biotechnol. Lett. **22,** 741–746.

Giordani, R.; Regli, P.; Buc, J., 2002: Antifungal effect of *Hevea brasiliensis* latex with various fungi. Its synergistic action with amphotericin B against *Candida albicans*. Mycoses **45,** 476–481.

Green, V. S.; Stott, D. E.; Diack, M., 2006: Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biol. Biochem. **38,** 693–701.

Güsewell, S.; Freeman, C., 2005: Nutrient limitation and enzyme activities during litter decomposition of nine wetland species in relation to litter N : P ratios. Funct. Ecol. **19,** 582–593.

Hernes, P. J.; Hedges, J. I., 2004: Tannin signatures of barks, needles, leaves, cones, and wood at the molecular level. Geochim. Cosmochim. Acta **68,** 1293–1307.

Jones, C. G.; Lawton, J. H.; Shachak, M., 1994: Organisms as ecosystem engineers. Oikos **69,** 373–386.

Kandeler, E.; Stemmer, M.; Klimanek, E. M., 1999: Response of soil microbial biomass urease and xylanase within particle size fractions to long-term soil management. Soil Biol. Biochem. **31,** 261–273.

Kanokwiroon, K.; Teanpaisan, R.; Wititsuwannakul, D.; Hooper, A. B.; Wititsuwannakul, R., 2008: Antimicrobial activity of a protein purified from the latex of *Hevea brasiliensis* on oral microorganisms. Mycoses **51,** 301–307.

Kosola, K. R.; Dickmann, D. I.; Hall, R. B.; Workmaster, B. A. A., 2004: Cottonwood growth rate and fine root condensed tannin concentration. Tree Physiol. **24,** 1063–1068.

Kourtev, P. S.; Ehrenfeld, J. G.; Huang, W. Z., 2002: Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. Soil Biol. Biochem. **34,** 325–332.

Kraus, T. E. C.; Zasoski, R. J.; Dahlgren, R. A., 2004: Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. Plant Soil **262,** 95–109.

Leon, M. C. C.; Stone, V.; Dick, R. P., 2006: Organic soil amendments: impacts on snap bean common root rot (*Aphanomyes euteiches*) and soil quality. Appl. Soil Ecol. **31,** 199–210.

Li, Y. T.; Rouland, C.; Benedetti, M.; Li, F. B.; Pando, A.; Lavelle, P.; Dai, J., 2009: Microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. Soil Biol. Biochem. **41,** 969–977.

Martin, M. N., 1991: The latex of *Hevea brasiliensis* contains high levels of both chitinases and chitinases/lysozymes. Plant Physiol. **95,** 469–476.

Mauch, F.; Mauch-Mani, B.; Bolier, T., 1988: Antifungal hydrolases in pea tissue II. Inhibition of fungal growth by combinations of chitinase and A-1,3-glucanase. Plant Physiol. **88,** 936–942.

Mora, P.; Miambi, E.; Jiménez, J. J.; Decaëns, T.; Rouland, C., 2005: Functional complement of biogenic structures produced by earthworms, termites and ants in the neotropical savannas. Soil Biol. Biochem. **37,** 1043–1048.

Nandris, D.; Chrestin, H.; Noirot, M.; Nicole, M.; Thouvenel, J. C.; Rio, B.; Geiger, J. P., 1991a: La nécrose du phloème du tronc de l'hévéa en Côte d'Ivoire : 1- Symptomatologie et caractéristiques biochimiques. Eur. J. For. Path. **21,** 325–339.

Nandris, D.; Thouvenel, J. C.; Chrestin, H.; Nicole, M.; Gianotti, J.; Déclert, C.; Rio, B.; Noirot, M., 1991b: La nécrose du phloème du tronc de l'hévéa en Côte d'Ivoire : 2- Etiologie de la maladie. Eur. J. For. Path. **21,** 340–353.

Nandris, D.; Moreau, R.; Pellegrin, F.; Chrestin, H., 2004: Rubber tree (*Heave brasiliensis*) bark necrosis syndrome II: first comprehensive report on causal stresses. Plant Dis. **88,** 1047.

Nandris, D.; Moreau, R.; Pellegrin, F.; Chrestin, H., 2005: Rubber tree bark necrosis: advances in symptomatology, etiology, epidemiology and causal factors of a physiological trunk disease. Trop. Agric. Sci. Technol. **28,** 1–8.

Nandris, D.; Moreau, R.; De Fay, E.; Masson, A.; Pellegrin, F.; Chrestin, H., 2006: Etiological investigations on trunk phloem necrosis of the rubber tree: biotic or abiotic causal factors? In: Tapping Panel Dryness of Rubber Trees. Ed. by Jacob, J.; Krishnakumar, R., Mathew, N. M. Kerala: Rubber Research Institute of India, pp. 85–92.

Naseby, D. C.; Moënne-Loccoz, Y.; Powell, J.; O'gara, F.; Lynch, J. M., 1998: Soil enzyme activities in the rhizosphere of field-grown sugar beet inoculated with the biocontrol agent *Pseudomonas fluorescens* F113. Biol. Fertil. Soil **27,** 39–43.

Nelson, N., 1944: A photometric adaptation of Somogyi method for the determination of glucose. J. Biol. Chem. **153,** 375–381.

Nicole, M.; Thouvenel, J. C.; Giannotti, J.; Chrestin, H.; Geiger, J. P.; Nandris, D.; Rio, B., 1991: The histology of *Hevea brasiliensis* phloem necrosis. Eur. J. For. Path. **21,** 183–196.

Nierop, K. G. J.; Preston, C. M.; Verstraten, J. M., 2006: Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. A case study using four tannins. Soil Biol. Biochem. **38,** 2794–2802.

Parijs, J. V.; Broekaert, W. F.; Goldstein, I. J.; Peumans, W. J., 1991: Hevein: an antifungal protein from rubber-tree (*Hevea brasiliensis*) latex. Planta **183,** 258–264.

Pellegrin, F.; Duran-Vila, N.; Van Munster, M.; Nandris, D., 2007: Rubber tree (*Hevea brasiliensis*) trunk phloem necrosis: aetiological investigations failed to confirm any biotic causal agent. For. Path. **37,** 9–21.

PEYRARD, N.; PELLEGRIN, F.; CHADOEUF, J.; NANDRIS, D., 2006: Statistical analysis of the spatio-temporal dynamic of rubber tree (*Hevea brasiliensis*) trunk phloem necrosis: no evidence of pathogen transmission. For. Path. **36,** 360–371.

RASMUSSEN, P. H.; KNUDSEN, I. M. B.; ELMHOLT, S.; JENSEN, D. F., 2002: Relationship between soil cellulolytic activity and suppression of seedling blight of barley in arable soil. App. Soil Ecol. **19,** 91–96.

RUAN, H.; LI, Y.; ZOU, X., 2005: Soil communities and plant litter decomposition as influenced by forest debris: variation across tropical riparian and upland sites. Pedobiologia **49,** 529–538.

SANNINO, F.; GIANFREDA, L., 2001: Pesticide influence on soil enzymatic activities. Chemosphere **45,** 417–425.

SANTOS, I. S.; CUNHAB, M. D.; MACHADOC, O. L. T.; GOMESA, V. M., 2004: A chitinase from *Adenanthera pavonina* L. seeds: purification, characterisation and immunolocalisation. Plant Sci. **167,** 1203–1210.

SCALBERT, A., 1991: Antimicrobial properties of tannins. Phytochemistry **30,** 3875–3883.

SOMOGYI, M., 1945: A new reagent for the determination of sugar. J. Biol. Chem. **160,** 61–68.

THIOULOUSE, J.; CHESSEL, D.; DODELEC, S.; OLIVIER, J. M., 1997: ADE-4: A multivariate analysis and graphical display software. Stat. Comp. **7,** 75–83.

VELASQUEZ, E.; PELOSI, C.; BRUNET, D.; GRIMALDI, M.; MARTINS, M.; RENDEIRO, A. C.; BARRIOS, E.; LAVELLE, P., 2007: This ped is my ped : visual separation and near infrared spectra allow determination of the origins of soil macroaggregates. Pedobiologia **51,** 75–87.

VEPSÄLÄINEN, M.; KUKKONEN, S.; VESTBERG, M.; SIRVIÖ, H.; NIEMI, R. M., 2001: Application of soil enzyme activity test kit in a field experiment. Soil Biol. Biochem. **33,** 1665–1672.

WURZBURGER, N.; HENDRICK, R. L., 2007: Rhododendron thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. Pedobiologia **50,** 563–576.

Conclusion

The principal component analysis carried out in this study showed for the first time that some biological activity indicators can help to distinguish between healthy trees (H and HH) and trees affected by TPN (B and BH). This distinction seems to be useful for observing the impact of TPN on the ecology of rubber tree plantations. Termite density was strongly associated with TPN (B and BH). High cellulase, xylanase and amylase activities were associated with healthy trees and were lower near diseased trees. PCA also indicated that *N*-acetyl-glucosaminidase activity was associated with the zone between healthy and affected trees (BH). It would seem that termite density, polysaccharidase activities and *N*-acetyl-glucosaminidase activity can be used as an indicator of the impact of TPN on soil ecology. However, the production of secondary metabolites as a result of TPN must be investigated. Further research needs to be carried out to assess the biological activities during the dry season in order to confirm this hypothesis.

III.1.5.2 Dry season

The expression of soil enzyme activities in dry season was showed in table 6. Enzyme activities were higher in soil under midway between trees (BH and HH) than under near collar of trees (B and H).

Polysaccharidases

Cellulase and xylanase activities under TPN trees (B and BH) were higher than under healthy trees (H and HH) and were the highest in midway between TPN and healthy trees (BH). Amylase activity was the highest in midway between healthy trees (HH) and was the lowest in near collar of TPN affected tree (B). However, no statistically significant difference in polysaccharidase activities was observed.

Heterosidases

β-glucosidase activity was higher in soil under midway between trees (BH and HH) than under near the collar of tree (B and H). This enzyme activity in soil under midway between healthy trees (HH) was the highest and soil under near collar of TPN tree (B) was the lowest. However, no significant difference was observed.

The assay of N-acetyl-glucosaminidase activity showed that the soils under midway between trees (HH and BH) were significantly higher ($P<0.05$) than under near collar of tree (B and H). Soil under midway between healthy trees (HH) was higher N-acetyl-glucosaminidase activity than under midway between TPN and healthy trees (BH).

Urease

Soil under midway between healthy trees (HH) showed the highest urease activity and it was significantly higher ($P<0.05$) than other zones.

Fluorescein diacetate (FDA) hydrolysis

Soil under midway between TPN and healthy trees (BH) was the highest FDA hydrolysis activity and was significantly higher ($P<0.05$) than under near collar of trees (B and H).

Table 6 Soil enzyme activities (μg of product g^{-1} soil h^{-1}) in *H. brasiliensis* plantation under four different zones; near collar of tree affected by TPN (B), midway between tree affected by TPN and healthy tree (BH), near collar of healthy tree (H) and midway between healthy trees (HH) during dry season

	B	**BH**	**H**	**HH**
Cellulase	2.1±2.2 a	2.4±1.6 a	2.0±0.6 a	1.8±1.5 a
Xylanase	0.9±1.5 a	1.2±1.2 a	0.0±0.1 a	0.8±1.0 a
Amylase	2.1±2.7 a	4.1±1.4 a	3.9±5.9 a	5.6±1.7 a
β-glucosidase	10.0±7.6 a	25.1±26.4 a	14.4±19.2 a	34.0±27.0 a
N-acetyl-glucosaminidase	3.4±1.3 a	6.9±2.0 b	2.6±1.5 a	7.2±2.0 b
Urease	1.2±0.5 a	2.0±0.6 a	1.5±0.3 a	3.2±1.4 b
FDA hydrolysis	0.8±0.2 ab	1.3±0.4 c	0.7±0.3 a	1.2±0.5 bc

Means within the same column followed by contrasting letters are significantly different among treatment using TukeyHSD test ($P<0.05$) (±: standard deviation, n=9)

Multivariate analysis

PCA showed axis 1 and axis 2 for 55.3% and 17.5% of the variance, respectively (Figure 6). Testing the significance of groupings (zones) was carried out on 10,000 permutations and was significantly discriminated (P<0.002). The PCA revealed that the soil under near collar of healthy tree (H) was associated with the low expression of all tested enzymes. The high values of urease, β-glucosidase, amylase, N-acetyl-glucosaminidase and FDA hydrolysis activities were associated with soil under midway between trees (BH and HH). The soil under midway between TPN affected tree and healthy trees (BH) had the highest expression of xylanase and cellulase.

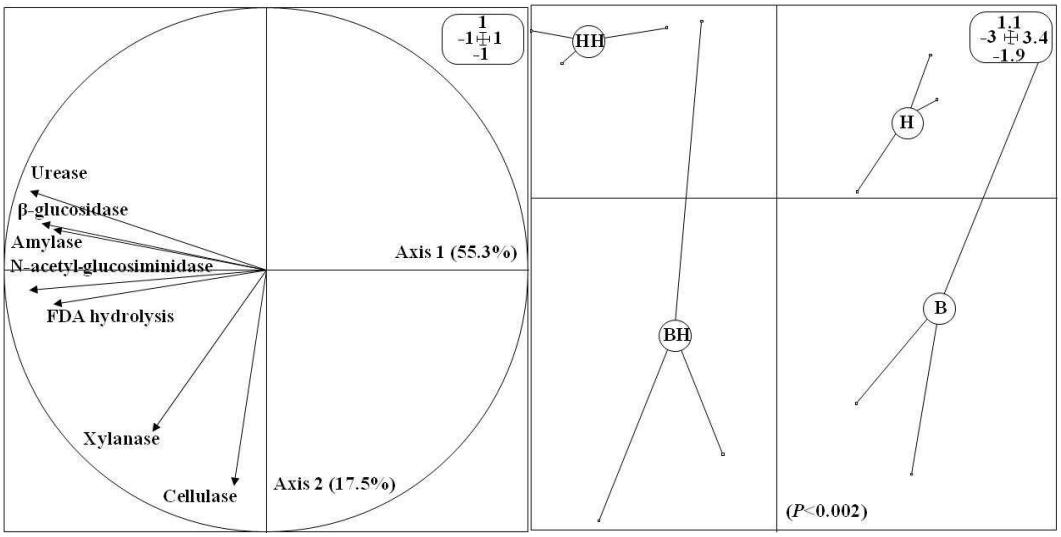

Figure 6 Principal component analysis showed the relationship between soil enzyme activities and sampling zones with near collar of TPN affected tree (B), near collar of healthy tree (H), midway between TPN affected tree and healthy tree (BH) and midway between healthy trees (HH) in dry season.

Conclusion

In contrast with rainy season, in dry season, PCA showed that xylanase and cellulase activities in soil with TPN affected trees (BH and B) were higher than with healthy trees (HH and H). This indicated that soil enzyme activities may be not greatly affected by tannins. At the time of taken samples, the *H. brasiliensis* shed leaves and is not collected latex, the trees are not stressed by tapping and thus, the tannins may be not stimulated. In addition, the concentration of soil tannins in dry season may be not enough to inhibit enzyme activities because tannins, the water soluble polyphenols, are readily leached and dispersed into soil by rainfall (Hättenschwiler and Vitousek, 2000; Teklay, 2004).

III.1.6 TPN AND FUNGAL COMMUNITY

These results have been submitted to “Environmental microbiology”.

The effect of trunk phloem necrosis on the structure of the fungal community in soil under rubber trees (*Hevea brasiliensis*)

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Summary

Trunk Phloem Necrosis (TPN) is an economically important disease of the rubber tree (*Hevea brasiliensis*) because it obstructs the latex flow. TPN is considered to be a physiological plant disorder caused by a combination of exogenous and endogenous stresses. Environmental stresses can affect the soil ecosystem and may also include plant stress. This study of the fungal community composition was carried out on soil in rubber plantations with a high incidence of trees affected by TPN to obtain better understanding of the impact of TPN on soil fungi. The analysis of culturable soil fungal populations revealed that the fungal density near trees affected by TPN was not significantly different from that near healthy trees. The analysis of the fungal diversity using a molecular approach (fungal 28S DNA-DGGE) with DNA extracted directly from the soil showed that fungal diversity near trees affected by TPN tended to be higher than near healthy trees. The structure of the soil fungal community was determined from a similarity dendrogram of 28S rDNA-DGGE banding patterns. There were two distinct clusters: near trees affected by TPN and near healthy trees. This suggested that there was a significant correlation between certain fungi and trees affected by TPN. The effect of TPN on soil fungi is discussed

Keywords: Rubber tree; Trunk phloem necrosis; Plant physiological disorder; fungal community.

Introduction

The rubber tree (*Hevea brasiliensis*) is an important source of industrial rubber. However, rubber tree trunk phloem necrosis (TPN) has become a major problem as it prevents latex production, which has had a serious effect on the economy. TPN is considered to be a plant disease caused by a physiological disorder as some etiologists have reported that it was not possible to detect biotic pathogens using either conventional (Peyrard *et al.,* 2006) or molecular methods (Pellegrin *et al.,* 2007). Exogenous and endogenous stresses may cause this syndrome (Nandris *et al.,* 2004) and stressed trees may also affect the soil ecosystem. Interaction between plants and microorganisms is an important part of a forest soil ecosystem. Soil quality depends to a considerable extent on soil microbes which are of fundamental importance for soil functioning and fertility (Elliott *et al.,* 1996). Soil microbiological parameters can, therefore, be used as indicators of disturbance to soil ecology (Dick, 1992; Dilly and Blume, 1998).

Fungi are heterotrophic organisms which play a role in soil nutrient cycling, because their extracellular enzymes degrade the organic matter that is the source of nutrients for them as well as for plants (Khaid *et al.,* 2006; Pavarina *et al.,* 1999; Tribak *et al.,* 2002; Vidal *et al.,* 1999). However, the decomposition capacity of fungi depends on the type and abundance of organic materials (Saliha *et al.,* 2005). Some fungi are able to convert toxic chemicals (such as cyanide) in soil into essential nutrients for plants (Maier-Greiner *et al.,* 1991). Fungi can also regulate soil physical properties as the stability of soil aggregates is affected by fungal hyphae (Harris *et al.,* 1964; TonThat and Cochran, 2000): this can assist water movement in soil and encourage root growth. Soil fungal diversity is influenced by various factors including the soil management system (Lucasa *et al.,* 2007), climate change (Freya *et al.,* 2008) and soil pollution (Kandeler *et al.,* 2000). The structure of the soil fungal community is, therefore, considered to be important for understanding the relationship between environmental factors and ecosystem functioning.

The effect of various factors on the composition of the soil microbial community can be studied using culture dependent and culture independent techniques. However, culture dependent methods still have limitations because many microorganisms in the soil cannot be cultured in synthetic media in laboratory conditions (Borneman *et al.,* 1996; Torsvik *et al.,* 1998; van Elsas *et al.,* 2000). Culture independent methods have been developed to study these microorganisms in their environment. Molecular biology techniques are effective for detecting both culturable and non-culturable microorganisms in the microbial populations present in soil. Denaturing gradient gel electrophoresis (DGGE) has been used widely to study the soil microbial community. This technique involves amplifying the target-DNA region and PCR products of similar length are separated in polyacrylamide gel containing a gradient of denaturing conditions (Kirk *et al.,* 2004; Nakatsu, 2007). The DGGE technique is a reliable, reproducible and rapid method for studying soil microbial diversity (Duineveld *et al.,* 2001; Maarit-Niemi *et al.,* 2001). This technique is used for studying the soil microbial communities associated with agricultural practices (Hagn *et al.,* 2003; Nakatsu *et al.,* 2000; Sekiguchi *et al.,* 2008; Siddique *et al.,* 2005), degradation substrates (Cosgrove *et al.,* 2007; Das *et al.,* 2007; van der Wal *et al.,* 2006) and plant genotypes (Ikeda *et al.,* 2006)

There is currently no information on how the soil fungal community responds to TPN. However, an examination of this process would be useful to show how trees affected by TPN influence soil fungi as this may have a long-term effect on soil ecology. This study was designed to provide information on the fungal community structure in soil under rubber trees affected by TPN, using both culture dependent and molecular approaches.

2. Methods and materials

2.1 Field site description

The field site was a 9 year-old rubber plantation in Buri Rum province, north east Thailand (15° 16′ 18″ N and 103° 0′ 6″ E). The plantation was planted with trees of variety PRIM600 and had a high incidence of TPN (20-25% of trees affected). The climate is tropical with a mean annual temperature of 26°C and mean annual rainfall 1207 mm (very low for a rubber plantation). The soil was sandy loam (67% sand, 24% silt and 9% clay) with low organic matter content (0.88-1.34%).

2.2 Soil sampling design and preparation

The soil samples were collected during the rainy season (July to October 2006). Trees affected by TPN and healthy trees were selected using the annual tree health survey carried out by D. Nandris. Ten plots were chosen and samples were taken from four different zones in each plot: near to trees affected by TPN (B), half-way between affected trees and healthy trees (BH), near to healthy trees (H) and half-way between healthy trees (HH). In each zone, a block of soil (12x12x10 cm) was taken and separated into three layers (0-2 cm, 2-5 cm, and 5-10 cm). The soil was air-dried, ground and sieved to 2 mm. Plant residues and roots were removed. The samples were prepared for fungal analysis by mixing soil from the same depth in three identical zones.

2.3 Soil fungal enumeration and isolation

The soil fungi were counted using a plate dilution method adapted from Diouf *et al.* (2005). The soil (5g) was agitated in 50 ml of dispersing solution (6g/l of sodium pyrophosphate and 1.2 g/l of bactopeptone) for 30 minutes and allowed to precipitate for 15 minutes. The fungal suspensions were diluted to 10% in a physiological solution (9g/1 of NaCl). 200 µl of each diluted fungal sample was cultured on Sabouraud medium (with 0.005% w/v of Chloramphenicol – Sigma). The assay was performed in triplicate. The size of fungal colonies was determined after incubation at 27°C for 5 days. The distinct colony morphotypes were isolated and purified on Sabouraud medium. The mycelia of selected fungi were conserved at -20 °C for molecular identification.

2.4 Identification of culturable fungi

The taxonomic position of selected fungi was identified by molecular biology. DNA was extracted from the mycelium using the method described by Di Battista (Di Battista,

1997). A 650 bp nucleotide fragment (ITS1-5.8S-ITS2 region) was amplified by PCR using primer sets ITS1 (5'TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White *et al.*, 1990). PCR amplification was performed using Ready-To-Go Taq poloymerease (Pharmacia). The PCR cycles were initial denaturation at 94°C for 5 minutes, 29 cycles at 94°C for 45 seconds, 60°C for 45 seconds, 72°C for 1 minute and a final elongation step at 72°C for 30 seconds using GenAmp PCR System 2400 (Perkin-Elmer). All PCR products were fingerprinted by electrophoresis using 5 µl of product in 2% (w/v) agarose gel with a 0.5X TAE buffer (Biorad) and ethidium bromide (0.5 µg/ml) staining. DNA sequencing was performed using Genome Express (Grenoble, France). The DNA sequences were compared with other DNA sequences using GenBank BLAST searches.

2.5 Genetic diversity of soil fungi

Fungal DNA was extracted from soil samples as described by Porteus (Porteous *et al.*, 1997). The 28S rDNA region was amplified using primer sets 403f (5' GTGAAATTGTTGAAAGGGAA 3') and 662r (5' GACTCCTTGGTCCGTGTT 3') with a 40 bp GC clamp (Sigler and Turcob, 2002). PCR amplification was performed using Ready-To-Go Taq poloymerease (Pharmacia). The cycles were initial denaturation at 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 50°C for 1 minute, 72°C for 2 minutes and a final elongation step at 72°C for 10 minutes using a GenAmp PCR System 2400 (Perkin-Elmer). The PCR products were fingerprinted as described above and PCR amplicons were determined for different positions by DGGE (Denaturing Gradient Gel Electrophoresis) using the DcodeTM Universal Mutation Detection System (Biorad, Richmond, CA). For DGGE, 20 µl of product was loaded onto 8% (w/v) polyacrelamide gel with a linear gradient of urea and formamide denaturants increasing from 35%-60% (100% denaturant contains 7 M urea and 40% (v/v) formaminde). Gel electrophoresis was carried out at 20V for 10 minutes then 75V for 16 hours. Gels were run in 0.5X TAE at a constant temperature of 60°C. The gels were stained using an ethidium bromide solution and photographed under UV transillumination using GelDoc 2000 system (Biorad).

2.6 Statistical analyses

The mean soil fungal densities in the four different zones were compared and the statistical difference was measured by the ANOVA-Tukey test using the R program version 2.7.0. The 28S rDNA DGGE banding patterns were analyzed based on the band appearance

and disappearance using image analysis software Quantity One (Bio-rad). Banding data from the DGGE gel was used to evaluate the genetic similarity of fungi from the four different sampling zones. The genetic similarity of fungal 28S rDNA was measured using Dice coefficients and a similarity dendrogram was created using UPGMA (Unweighted Pair Group Method of Arithmetic means) and Quantity One, version 4.2.1.

3. Results

3.1 Soil fungal enumeration and selected strains

The fungal community was evaluated using a plate dilution method. The number of culturable fungal strains in zone H were higher than in zone B but no significant difference in fungal density was observed. However, the number of fungal strains in zone BH was slightly higher than in zone HH but, again, there was no significant difference in fungal density (Table 1).

Five fungal strains were selected based on their abundance in the soil under trees affected by TPN (B and BH). The selected fungi were identified by comparing the DNA sequence with the Genbank database using the Blast program. This revealed that *Paecilomyces lilacinus* and *Thielavia hyrcaniae* were dominant in zone BH while *Trichoderma asperellum* and *Corynascus kuwaitiensis* were dominant in zone B (Table 2).

Table 1. Number and density of fungi in soil in near the collars of trees affected by TPN (B), midway between diseased trees and healthy trees (BH), near the collars of healthy trees (H) and midway between healthy trees (HH).

Sampling zones	Number of fungal strains	Density (Log_{10} CFU/g of dry weight soil)
B	25	3.46 ±0.22 a
BH	22	3.33 ±0.27 a
H	30	3.55 ±0.22 a
HH	19	3.40 ±0.20 a

Means within the same row followed by different letters are significantly different using Tukey HSD test ($P<0.05$) (±: standard deviation, n=9)

Table 2. Proportion of each selected fungal strains out of the total number of fungi and their taxonomic position by matching ITS regional sequence in GenBank

Strain	Proportion of total number of fungi (%)*				Referenced in GenBank	Similarity (%)
	B	BH	H	HH		
P	27	32	0	14	*Paecilomyces lilacinus* (AB103380)	100
C	4	0	1	0	*Corynascus kuwaitiensis* (AJ715483)	90
Th	3	12	0	0	*Thielavia hyrcaniae* (AJ271580)	99
Tr1	13	0	0	0	*Trichoderma asperellum* (EU280110)	91
Tr2	12	4	0	0	*Trichoderma asperellum* (EU280110)	99

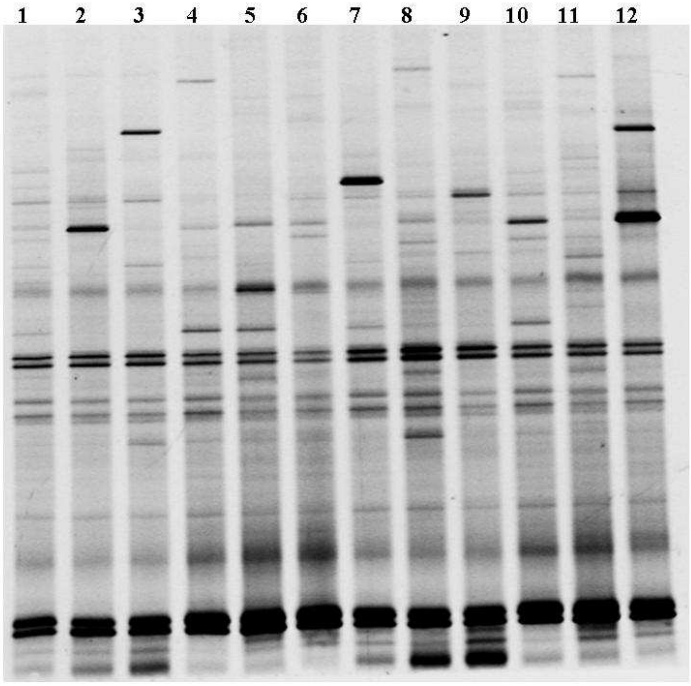
*The proportion of selected fungi near the collars of diseased trees (B), midway between diseased trees and healthy trees (BH), near the collars of healthy trees (H) and midway between healthy trees.

3.2 Genetic diversity of soil fungi

The total DNA was extracted directly from the soil under rubber trees and was pure enough for PCR amplification of 28S rDNA fragments using primers 403f and 662r. The PCR products were separated by DGGE so that the genetic diversity in soil near trees

affected by TPN (B and BH) and healthy trees (H and HH) could be compared. The DGGE banding patterns were obtained by migrating PCR products in gel. The DGGE patterns in each soil layer showed that the fungal diversity differed slightly between the zones (Fig. 1). The DGGE profiles of soil at a depth of 0-5 cm showed that the genetic diversity of fungi in zone B (37 bands) was higher than in zone H (33 bands). The fungal soil in zone BH (31 bands) had greater genotypic diversity than in zone HH (27 bands) (Fig. 1a). The DGGE profiles of soil at a depth of 5-10 cm revealed that the genetic diversity in trees affected by TPN (34 bands in zone B and 30 in zone BH) were higher than in healthy trees (28 bands in zone H and 29 in zone HH) (Fig. 1b). The DGGE profiles of soil at depths of both 0-5 cm and 5-10 cm showed that some bands of PCR products had migrated a similar distance in all zones, indicating the presence of soil fungi common to all zones.

a



b

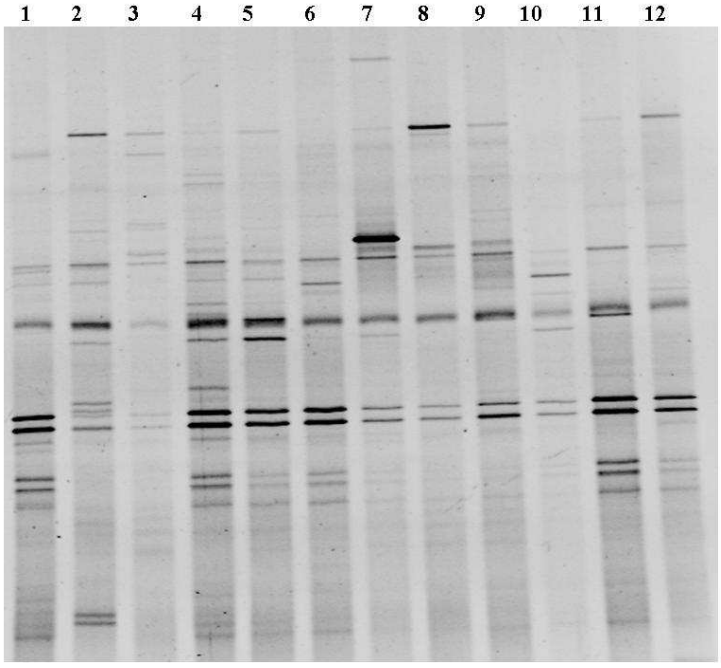
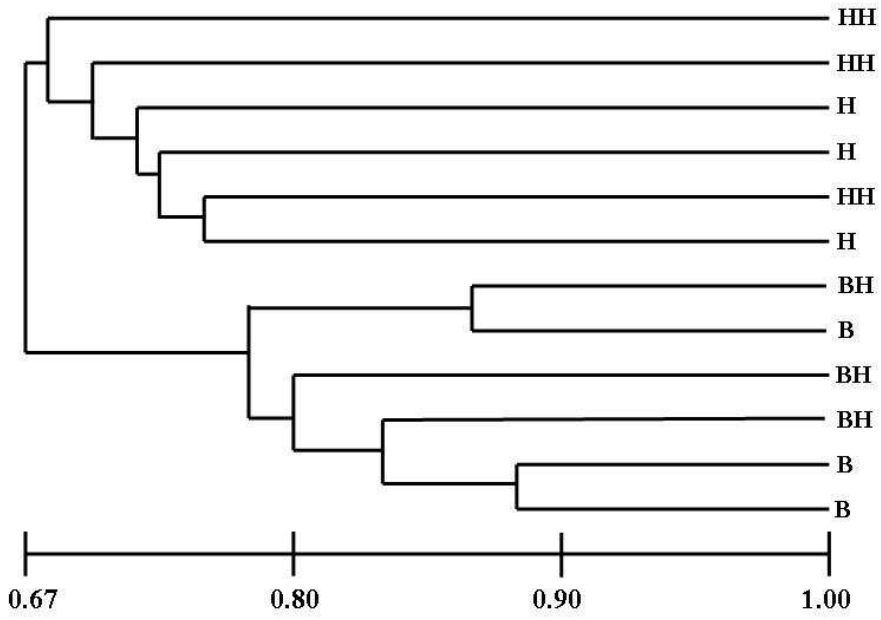


Fig. 1. Denaturing gradient gel electrophoresis (DGGE) banding profiles of fungal 28S rDNA from soil at a depth of 0-5 cm (a) and 5-10 cm (b) near the collars of trees affected by TPN (lanes 1-3), midway between diseased trees and healthy trees (lanes 4-6), near the collars of healthy trees (lanes 7-9) and midway between healthy rubber trees (lanes 10-12).

a



b

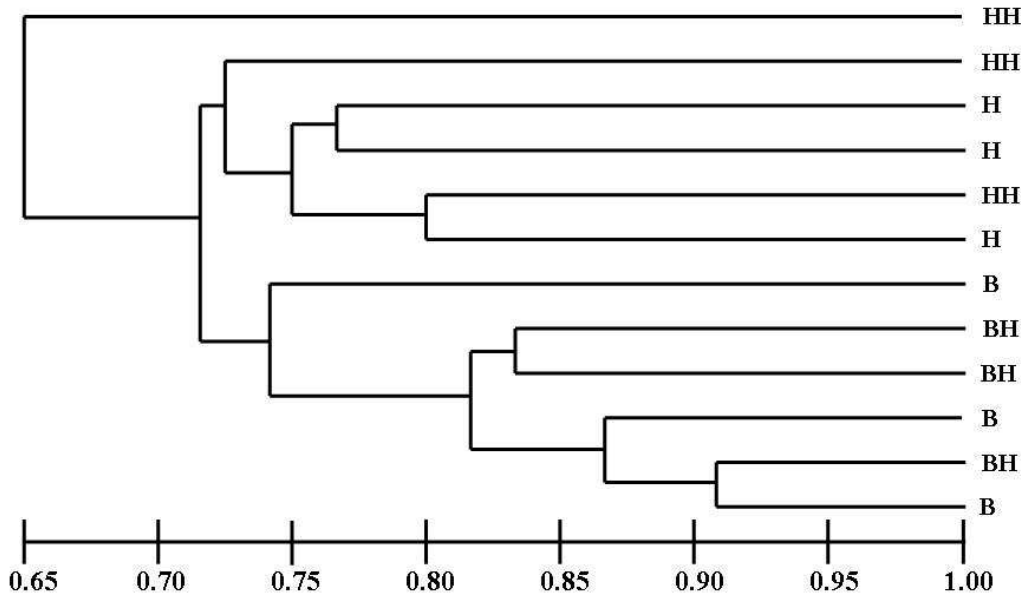


Fig. 3. The similarity dendrograms of the 28S rDNA-DGGE banding profiles of four different zones (B: near the collars of trees affected by TPN and BH: midway between diseased trees and healthy trees, H: near the collars of healthy trees, HH: midway between healthy trees) at a depth of 0-5 cm (a) and 5-10 cm (b). The dendrograms were constructed using Dice's coefficient and clustered by the unweighted pair group method using arithmetic averages (UPGMA).

3.3 Structure of soil fungal community

The cluster dendrogram was created by UPGMA using 28S rDNA-DGGE banding patterns. The dendrogram was used to describe the similarities between the soil fungal communities in four zones (B, BH, H and HH). The fungal communities near trees affected by TPN-trees were closer to each other than they were to those near healthy trees. The similarity dendrograms clearly showed two distinct clusters in soil at a depth of both 0-5 cm and 5-10 cm. The UPGMA dendrogram showed that the fungal community in soil near to trees affected by TPN (B and BH) was distinct from that near to healthy trees (H and HH) with 0.67 similarities for soil at a depth of 0-5 cm and 0.72 similarities for soil at a depth of 5-10 cm (Fig. 2). Clustering indicated that the composition of soil fungal communities near healthy trees was dissimilar from that near to trees affected by TPN.

4. Discussion

This is the first study on the composition of fungal community in soil in rubber plantations with a high incidence of TPN. The soil fungal community was used as a biological indicator of ecological change because it responds to environmental disturbance (Dick, 1992; Dilly and Blume, 1998; Houston *et al.,* 1998; Kasela *et al.,* 2008; Lodge, 1997; Persiani *et al.,* 1998). The fungal communities in soil at a depth of 0-10 cm under healthy trees (H and HH) and diseased trees (B and BH) were compared. The fungal populations in each zone were analyzed using the culture-dependent plate-counting method. Although fungal diversity was highest in zone H, the density was not significantly different from that in other zones. This may be due to the propagation capacity of fungal species. Some fungi such as *Paecilomyces* sp. and *Trichoderma* sp. can produce propagules rapidly and easily. The method of fungal enumeration used in this study may include the distribution of mycelia or spores and thus overestimate these species by comparison with others. However, in this case, the fungal diversity in areas with a low abundance of organic resources (B and H) was higher than in areas with a high abundance of resources (BH and HH). Because soil with abundant substrates (litter or dead plant materials) influences microbial growth by providing nutrient sources, microbial populations with high growth rate and activity can colonize quickly more than microbial populations with low growth rate and activity. In the absence of litter or dead plant material, some soil microorganisms have a high potential for seeking food. For instance, some fungi of the *Paecilomyces* genus are nematophagous (Bhat *et al.,* 2009; Carneiro and Cayrol, 1991) (Gaspard *et al.,* 1990; Hewlett *et al.,* 1988). Many studies have reported that some of the Trichoderma genus are parasitic on nematodes and fungi (Elad and Kapat, 1999;

Nagayama *et al.*, 2007; Sharon *et al.*, 2007). Fungi with a low colonization capacity and low activities may, therefore, be difficult to detect by the method used in this study. As the soil with lower resources was not adequate for microbial growth, the influence of fast growing fungi was less and so it was easy to detect fungal diversity. The same fungal diversity pattern was also found using the biological molecular approach.

The fungal diversity was analyzed using PCR-DGGE on DNA directly extracted from soil, amplifying the 28S rDNA region (approximately 260 bp). The 28S rDNA-DGGE fingerprints revealed that the diversity obtained by detecting ribotypes was higher than that obtained by detecting morphotypes. Some authors have reported the detection of unculturable fungi using DDGE (Bates and Garcia-Pichel, 2009; Bougoure and Cairney, 2005; Gao *et al.*, 2008; Li *et al.*, 2008; Meroth *et al.*, 2003). However, the results obtained using PCR-DGGE to study community complexity depends on the primer used (Green *et al.*, 2004; Marshall *et al.*, 2003; Zuccarob *et al.*, 2003). For evaluating soil fungi, using 28S rDNA as the target gives a higher diversity than 18S rDNA (Diouf *et al.*, 2005) and may provide higher resolution for DGGE (Bonanomi *et al.*, 2008; Möhlenhoff *et al.*, 2001; Zuccaro *et al.*, 2008).

The structure of the fungal community in soil (0-5 cm and 5-10 cm depth) obtained from similarity dendrogram of 28S rDNA-DGGE fingerprints showed that the fungal groups near healthy trees (H and HH) were clearly separated from those near diseased trees (B and BH). This indicated that some fungal strains in trees affected by TPN were different from those near healthy trees. The soil microbial community within the ecosystem may be affected by many stresses including changes in environment under abiotic conditions and changes in quality resources (Brussaard *et al.*, 2005). In this study, determining factors for the soil fungal community structure may arise from root exudates or litter component of trees. Root exudates are chemical compounds produced by plant roots which secrete into the surrounding rhizosphere. Root exudation can regulate the soil microbial community (Bais *et al.*, 2006; Walker *et al.*, 2003). The type and quantity of root exudates depend on many factors such as the plant species (Ström *et al.*, 1994; Zhang *et al.*, 2007), the plant varieties (Huang *et al.*, 2008; Rae and Castro, 1967) and the plant development stage (Aulakh *et al.*, 2001; Mougel *et al.*, 2006). The chemicals released by plant roots affect the soil fungal community by both increasing and decreasing the abundance of fungal species (Broeckling *et al.*, 2008; Kumar *et al.*, 2007). The root exudates from trees affected by TPN may induce some new fungal species in the rhizosphere. However, the differences in chemical compounds of root exudation from healthy trees and diseased trees have not yet been demonstrated.

The analysis of plant chemical components can identify plant stresses. Many woody plants respond to stresses by producing secondary metabolites such as tannins (Hernes and Hedges, 2004; Yu and Dahlgren, 2000) which are water soluble polyphenols(Spencer *et al.*, 1988). Tannins can be induced by several factors including abiotic factors (Carter *et al.*, 1999) and human factors (artificial xylem injury) (Eyles *et al.*, 2004). A recent study showed the effect of TPN on soil enzymatic activities: polysaccharidase activities including cellulase, xylanase and amylase were lower in soil under trees affected by TPN and suggested that tannins affect extracellular microbial enzymes by disrupting their activities (paper in press). Because TPN is a plant physiological disorder which may be caused by multiple stresses (Nandris *et al.*, 2004), a tree affected by TPN may produce tannin in at least one of its parts, such as bark, leaves or roots. Tannins do not reduce soil fungal populations (Baptist *et al.*, 2008) but the high tannin concentration produced by some plants can inhibit fungal growth (Nichols-Orians, 1991). However, this biochemical plant defense may induce new strains or abundance of fungi in soil under trees affected by TPN that can degrade tannin, indicating a difference from soil under healthy trees. The soil microbial community composition was found to be correlated to the concentration of tannin in the soil and fungi that have the ability to degrade tannin can adjust easily to a high concentration of phenol (Bhat *et al.*, 1997; Gamble *et al.*, 1996; Makkar *et al.*, 1993; Mutabaruka *et al.*, 2007; Pinto *et al.*, 2001). It has also been reported that some fungi in soil, such as fungal species in the *Paecilomyces* genus (Battestin and Macedo, 2007; Guedegbe *et al.*, 2008 ; 2009; Mahendran *et al.*, 2006) and *Trichoderma* (Bajpai and Patil, 1996; Bajpai and Patil, 1997) can produce enzymes to degrade tannins. These genera were dominant in soil near trees affected by TPN possibly because of their ability to tolerate the secondary metabolites of diseased trees. Furthermore, the area round diseased trees had a greater accumulation of wood from dead bark which also had a predominance of *Paecilomyces, Trichoderma, Thielavia* and *Corynascus*, these fungi being able to degrade plant residues (Eslyn *et al.*, 1975; Gerber *et al.*, 1997; Kluczek-Turpeinen *et al.*, 2003; Lopez *et al.*, 2006; Maheshwari *et al.*, 2000; Martínez *et al.*, 2005; Ryckeboer *et al.*, 2003) and also to resist or degrade polyphenol.

This study suggested that TPN affected the soil fungal community. The similarity dendrogram of 28S rDNA-DGGE explained the structure of the fungal community composition indicating that some fungi were positively correlated in the soil round trees affected by TPN. The secondary metabolites produced by diseased trees may affect fungal populations. Little is known about the interaction between biochemical plant defenses and

diseased trees and soil fungal populations. It would be useful to study the secondary metabolites generated by rubber trees affected by TPN.

5. Conclusions

The fungal UPGMA dendrogram of 28S rDNA-DGGE gel showed that the cluster of fungal populations in soil under trees affected by TPN was distinct from that under healthy trees. Although the fungal density in diseased trees was not significantly different from healthy trees, the number of fungal strains in diseased trees (BH) was slightly higher than in healthy trees (HH) using both culture-dependent and molecular approaches. This may indicate that some soil fungi in diseased trees were different from those in healthy trees. Some fungi including *Paecilomyces* sp. *Thielavia* sp. and *Trichoderma* sp. were dominant in soil under trees affected by TPN. These fungal species may be used as biological indicators of the impact of TPN on soil ecology. This study suggested that TPN may have a long-term effect on the fungal community.

III.1.7 DISCUSSION

III.1.7.1 TPN and soil properties

In this study, organic carbon, total N, C:N ratio and organic matter content were investigated for indicating status of soil fertility. The soil under midway between healthy trees (HH) had fertility higher than under midway between TPN affected tree and healthy trees (BH). However, no significant difference was observed. This may occur from the area under midway between TPN affected tree and healthy trees (BH) have only one TPN affected tree among healthy trees and thus, ratio of TPN affected tree and healthy tree is 1:4. In his ratio, the expression of impact of TPN disease may be not distinctly. Thus, the impact of TPN on soil biological activities and soil chemical properties was unclear. However, the impact of TPN on soil ecology may express in the rainy season more than in the dry season because the trees are continuously stressed by tapping during the rainy season. The TPN, caused of damaging phloem system, may directly affect translocation soluble organic materials to all parts of tree. When the phloem system is dysfunctional, the organic compounds in parts of tree (especially, litter) may be reduced. The litter quality depends on chemical component in leave (Bardgett and Shine, 1999; Warren and Zou, 2002; Abraham and Chudek, 2008). Thus, the litter quality of TPN affected tree may lower than healthy tree and consequently, soil fertility rather low in area under TPN affected tree.

III.1.7.2 Impact of trunk phloem necrosis on macrofauna community

The macrofauna diversity has used as biological indicator of soil disturbance because it reflects the impact of environmental change (Nahmani *et al.,* 2006; Velasquez *et al.,* 2007; Yankelevich *et al.,* 2007). In the rainy season, most invertebrates were associated with areas between trees (BH and HH) more than areas near collars of trees (B and H). The areas between trees in this plantation were accumulated with organic materials, especially, litter. The higher food source influences macrofauna population and diversity. In the rainy season, the difference in Coleoptera abundance between BH and HH was not obviously different but the diversity was the highest in area between healthy trees (HH). Likewise, the earthworm population was mostly found in soil under between healthy trees (HH). For the tropical forest, the quality of litter has been showed to affect macrofaunal population (Zou, 1993; Mboukou-Kimbatsa *et al.,* 1998; Gonzalez and Zou, 1999). The quality of plant materials depends on

chemical component which is the important factor to regulate the macrofaunal abundance and diversity. As mentioned above, the TPN affected tree may contain tannins more than healthy tree. The exceeding tannins can adversely affect some invertebrates. Tannins are binding protein agents, which are able to bind digestive enzymes to limit assimilation in insects. This has effect on survival and growth of macrofauna. Consequently, macrofaunal abundance and diversity was low (Loranger-Merciris *et al.*, 2007). However, (Ayres *et al.*, 1997) suggested that the same tannin had different effect on different herbivore insects. In this plantation, Coleoptera genus *Cerambycidae*, *Elateridae*, *Scarabaeidae* and *Dytiscidae* were not found in both soil and litter under TPN affected trees (B and BH). These Coleoptera genera may be affected by tannins from trees affected by TPN. On the other hand, some insect species in Carabidae and Tenebrionidae were detected in all sampling zones. These macrofaunas may resist polyphenols.

The termite density was higher under TPN affected trees (B and BH). Severe TPN causes the bark to slough off at the collar. As only healthy bark containing latex and cyanide is able to block boring insects, the unprotected wood can be attacked by termites to become part of their habitat. Consequently, the zones with the highest termite population were strongly associated with areas under the affected trees.

Soil macrofauna diversity in the dry season was lower than in the rainy season. The Coleoptera was detected in only soil under between TPN affected tree and health trees (BH) but its diversity was very low, only Tenebrionidae was found. This may be due to climate condition. The climate is considered to be the most important factor in the regulation of macrofauna community. In drought circumstance, soil was arid and hard because of decreasing soil water levels. Soil moisture, which is greatly associated with seasonal variation, significantly affects soil macrofauna (Sroka and Finch, 2006). In this season, no earthworm was found in all sampling zones because the earthworms maybe migrate to deeper soil layers. In during winter and summer, the earthworms can reach a depth of 40-45 cm and was in quiescent stage (Reddy and Pasha, 1993) whereas macrofauna was collected in a maximum depth of 30 cm in this study.

III.1.7.3 Relationship between trunk phloem necrosis and soil enzyme activity

Principal component analysis (PCA) revealed that most of enzyme activities were low in soil under near collars of tree (H and B). These sampling zones did not accumulate leaf litter, which is a major source of soil organic matter. Plant litter is an important source of

energy for soil microorganisms (Bardgett and Shine, 1999; Ruan *et al.,* 2005; Abraham and Chudek, 2008) which mainly produce soil enzymes (de Caire *et al.,* 2000; Stark *et al.,* 2008). In contrast, most of enzyme activities were high in soil under midway between trees (HH and BH). These zones were accumulated with abundant litter and had high humidity and thus the environmental conditions stimulated producing soil enzymes. There has been reported that the soil with litter addition can increase soil microbial activities (Dornbush, 2007).

In the rainy season, cellulase, xylanase and amylase activities were higher in soil under zones of healthy trees (H and HH) than under zones of TPN affected trees (B and BH). Thus, soils with healthy trees were strongly associated with polysaccharidases, which can break down carbohydrates and release glucose as a source of available energy for plants and microorganisms. On the other hand, the polysaccharidase activities were low in soils with TPN affected trees. This result may be due to the difference in chemical composition of plant materials between healthy and TPN affected trees. This difference may have an impact on the quality of litter and could, therefore, explain the differences in soil enzyme activities. Numerous studies revealed that soil enzyme activities depend on organic input. The litter quality influences various enzyme activities in litter decomposition and also results in increasing or decreasing the litter decomposition in soil (Fioretto *et al.,* 2000; Kourtev *et al.,* 2002; Güsewell and Freeman, 2005). In *H. brasiliensis* plantation in this study, dead plant materials or plant components (such as bark, braches, litter and latex) were the main source of organic matter decomposition. However, TPN affected tree may produce defensive chemicals and the accumulation of these chemicals in plant components may affect soil enzyme activities. Plant defensive chemicals are secondary metabolites, which are produced when plants suffer from stresses, e.g., pathogenic attack, adverse environmental factors, and mechanical and chemical injuries.

Some papers have reported that TPN may be caused by multiple exogenous and endogenous stresses (Nandris *et al.,* 2004; 2005; Nandris *et al.,* 2006). Tapping bark for latex collection (too frequently, badly carried out or using chemicals for latex stimulation) can conduce the cumulative stress of tree. Rubber tree can respond to such wounds or stresses by producing defensive chemicals including polyphenols and chitinase. However, some plant defensive chemicals, such as tannins, can disrupt enzyme activities which occur during the decomposition of organic input. Tannins are secondary plant metabolites, found in higher plant including woody plants, in any parts of plant such as leaves (Yu and Dahlgren, 2000), bark (Matthews *et al.,* 1997; Hernes and Hedges, 2004) and root (Peterson *et al.,* 1999; Kosola *et al.,* 2004). Many forest plants produce large quantities of tannin in the litter (Kraus

et al., 2003). Tannins in plants can be induced by many factors and they have been shown to be defense mechanisms against pathogens (Hewlett *et al.,* 1997) herbivores (Coley and Barone, 1996; Ayres *et al.,* 1997; Nomura and Itioka, 2002), abiotic factors (Carter *et al.,* 1999) and human factors; tannin was detected in a wound in eucalyptus caused by an artificial xylem injury (Eyles *et al.,* 2004).

Tannins are water soluble polyphenols that can precipitate proteins (Spencer *et al.,* 1988). Tannins can inhibit the activity of extracellular microbial enzymes (Scalbert, 1991) including polysaccharidases such as cellulase and xylanase (Gamble *et al.,* 2000; Barahona *et al.,* 2006). Both the quality and quantity of litter tannins can cause enzyme inhibition or reduce enzyme expression in soil (Joanisse *et al.,* 2007) and high concentration of tannins in leaves appears to inhibit decomposition (Driebe and Whitham, 2000; Lorenz *et al.,* 2000). It was reported that tannins are readily transferred to the soil by leaching from litter (Schofield *et al.,* 1998). Furthermore, high levels of tannins may influence the decomposition of soil organic matter by extracellular enzymes, soil mineralization, humus formation and decreasing soil fertility (Bradley *et al.,* 2000; Driebe and Whitham, 2000; Kraus *et al.,* 2004; Nierop *et al.,* 2006; Wurzburger and Hendrick, 2007).

PCA showed that N-acetyl-glucosaminidase activity was associated with area between TPN affected tree and healthy trees (BH). *N*-acetyl-glucosaminidase is one of chitinases that degrade chitin (Ekenler and Tabatabai, 2002). Some chitinase activity in soil may be produce by plants as a defensive mechanism. Plant chitinase has been found in plant tissues such as seed, bark, leaves and root (Albrecht *et al.,* 1994; Hietala *et al.,* 2004; Rakwal *et al.,* 2004; Santos *et al.,* 2004). This enzyme plays role of a chemical defense mechanism (Mauch *et al.,* 1988) and is rarely detectable in healthy plants but can be produced in reaction to damage or stress caused by various biotic or abiotic factors. Some studies have showed that *H. brasiliensis* can produce chitinase (Bokma *et al.,* 2002). Hevein, a chitinase from *H. brasiliensis* (Gidrol *et al.,* 1994), has been showed to be an antifungal agent inhibiting microorganisms (Giordani *et al.,* 2002; Kanokwiroon *et al.,* 2008) and several pathogenic fungi (Parijs *et al.,* 1991). The chitinase level is high in laticifers producing latex but very low in healthy tissues such as leaves, stem and roots (Martin, 1991). However, chitinases in plants can increase rapidly when stimulated by phytopathogenic microorganisms, pest attacks and abiotic stress such as wounding (Clarke *et al.,* 1994; Clarke *et al.,* 1998; Hietala *et al.,* 2004; Rakwal *et al.,* 2004; Hartz *et al.,* 2005).

In contrast, in the dry season, PCA showed that xylanase and cellulase activities in soil with TPN affected trees (BH and B) were higher than with healthy trees (HH and H).

This indicated that soil enzyme activities may be not greatly affected by tannins. At the time of taken samples, the *H. brasiliensis* shed leaves and is not collected latex, the trees are not stressed by tapping and thus, the tannins may be not induced. In addition, the concentration of soil tannins in the dry season may be not enough to inhibit enzyme activities because tannins, the water soluble polyphenols, are readily leached and dispersed into soil by rainfall (Hättenschwiler and Vitousek, 2000; Teklay, 2004). Furthermore, the areas with TPN affected trees (B and BH) were high in species richness of Coleoptera, termites and ants. These macrofaunas are epigeic species, which comminute the plant materials (such as litter and bark). The comminuted materials are readily degraded by xylanase and cellulase. Meanwhile, PCA showed that amylase activity was rather high in soil under healthy trees. This may depend on plant carbohydrate metabolism, which can be influenced by stresses. Several biotic and abiotic stresses can change the concentration of carbohydrate in plants (Jeun and Hwang, 1991; Abdalla and El-Khoshiban, 2007; Morsy *et al.*, 2007). In this case, the TPN may involve with decreasing amylase synthesis of *H. brasiliensis*. Moreover, many fungi in soil can express the amylase activity (Domingues and Peralta, 1993). In this plantation, the numeration of fungi showed that the fungal diversity in soil under midway between healthy trees (HH) was the highest. This may result in the highest amylase activity in this sampling zone and the highest β-glucosidase and urease activities were also included.

This study shows that TPN of *H. brasiliensis* has strong long-term effects on the soil ecosystem and soil fertility. PCA showed that the soil under TPN affected trees has lower polysaccharidase activities in rainy season. This may be the result of secondary metabolites produced by affected trees. As little is known about the relationship between TPN and defensive chemicals and the impact of these on soil enzymes in *H. brasiliensis* plantation, research is required into the secondary metabolites of TPN affected tree.

III.1.7.4 The influence of trunk phloem necrosis on soil microbial community

In this study, the assessment of soil microbial community in rain season was concentrated because soil moisture greatly influences microbial activities and propagation. In addition, the result of soil enzymes assay indicated that soil enzyme activities were greatly affected by TPN in rainy season.

Soil fungal community has been used as biological indicator of ecological change because it can respond to environmental disturbance (Dick, 1992; Lodge, 1997; Dilly and Blume, 1998; Houston *et al.*, 1998; Persiani *et al.*, 1998; Kasela *et al.*, 2008).In this

plantation, the fungal community in soil at deep layer of 0-10 cm with healthy trees (H and HH) and TPN affected trees (B and BH) were compared. Each zone, the fungal community composition were analyzed by cultivation-dependent using plate-count method. Although the fungal density between soils under healthy trees (H and HH) and TPN affected trees (B and BH) were not significantly different, the fungal diversity in soil under near collar of healthy tree (H) was the highest. This result may be involved with soil sodicity because the sodium concentration levels adversely affect soil microbial community (Rietz and Haynes, 2003; Wong *et al.,* 2008). The soil under near collar of healthy tree (H) in rainy season was the lowest in sodium concentration whereas the fungal diversity was the highest. In contrast, the sodium concentration in this zone in dry season was the highest whereas fungal diversity was the lowest. Furthermore, no significant difference in fungal density may be due to capacity of fungal species in their propagation. Some fungi in soil such as *Paecilomyces* sp. and *Trichoderma* sp. can rapidly produce propagules. Thus using method of fungal enumeration in this study may consider the distribution of mycelia or spores and the overrating of these species were observed when compared with others. However, in this case, the fungal diversity in areas with low abundance of organic resources (B and H) was rather high than in areas with high abundance of resources (BH and HH). The soil with abundant substrates (litter or plant dead materials) greatly influences microbial growth in sense of theirs nutrient sources. In addition, soil moisture, which greatly affects microbial reproduction, was higher in soils under between trees (BH and HH) than in soil under near collars of trees (B and H). The microbial with high properties of growth rate and activity can colonize quickly more than others microbial with low properties of those. Except litter or plant dead materials, some soil microbial has high potential in seeking food. For instance, some fungal species in genus *Paecilomyces* are nematophagous (Carneiro and Cayrol, 1991; Bhat *et al.,* 2009) (Hewlett *et al.,* 1988; Gaspard *et al.,* 1990)which can feed nematodes as theirs nutrient. Several studies reveled that some strains in genus *Trichoderma* were characterized as parasitism on nematodes and fungi (Elad and Kapat, 1999; Nagayama *et al.,* 2007; Sharon *et al.,* 2007). Thus fungi with low capacity of colonization may be difficult to detect by using method in this study. In addition, slow growing fungi in soil with lower nutrient resources or moisture may be less affected by fast growing fungi because the soil condition is not suitable for fungal colonization and consequently the most culturable fungi was easily detected.

In this study, investigation of soil fungal diversity by biological molecular approach using PCR-DGGE technique was also included. The DNA directly extracted from soil and the region of 28S rDNA (approximately 260 bp) was amplified. The 28S rDNA-DGGE

fingerprints revealed the diversity obtained by detecting ribotypes was higher than diversity obtained by detecting morphotypes. Some authors reported that unculturable fungi were detected by DDGE technique (Meroth *et al.,* 2003; Bougoure and Cairney, 2005; Gao *et al.,* 2008; Li *et al.,* 2008; Bates and Garcia-Pichel, 2009). However, using PCR-DGGE technique for studying community complexity, the obtained results were various depend on primer used (Marshall *et al.,* 2003; Zuccarob *et al.,* 2003; Green *et al.,* 2004). To evaluate the soil fungal community, using 28S rDNA as the target had diversity higher than 18S rDNA (Diouf *et al.,* 2005) and may present the higher resolution for DGGE procedure (Möhlenhoff *et al.,* 2001; Bonanomi *et al.,* 2008; Zuccaro *et al.,* 2008).

The relationship between fungal community and sampling zones was investigated using similar dendrogram of 28S rDNA-DGGE fingerprints. The dendrogram revealed the fungal group in soil (both 0-5 cm and 5-10 cm) under healthy trees (H and HH) was clearly separated from under TPN affected trees (B and BH). This indicated that some fungal strains in soil under TPN affected trees may be different from under healthy trees. The soil microbial community within ecosystem can change by many stresses including changing environment under abiotic conditions and changing quality resource (Brussaard *et al.,* 2005). In this study, determining factors of soil fungal community structure may arise from root exudates or litter component. Root exudates are chemical compounds that produce by plant root and secrete into surrounding rhizosphere. Root exudation can regulate the soil microbial community (Walker *et al.,* 2003; Bais *et al.,* 2006). Type and quantity of root exudates depend on many factors such as plant species (Ström *et al.,* 1994; Zhang *et al.,* 2007), plant varieties (Rae and Castro, 1967; Huang *et al.,* 2008) and stage of plant development (Aulakh *et al.,* 2001; Mougel *et al.,* 2006). The releasing plant root chemistry can positively and negatively influences soil fungal species (Kumar *et al.,* 2007; Broeckling *et al.,* 2008). The root exudates by TPN affected tree may induce some fungal species in growth and reproduction. For instance, *Paecilomyces lilacinus, Thielavia hyrcaniae, Corynascus kuwaitiensis* and *Trichoderma asperellum* were abundant in soil under TPN affected trees. Nevertheless, the differences in chemical compounds of root exudation from healthy tree and TPN-tree have not yet been demonstrated.

Determination of plant chemistry component has related plant stresses. Numerous woody plants can respond to stresses by producing some of secondary metabolites such as tannins (Yu and Dahlgren, 2000; Hernes and Hedges, 2004) which are water soluble polyphenols(Spencer *et al.,* 1988). Tannins can be induced by several factors including abiotic factors (Carter *et al.,* 1999) and human factor: making artificial xylem injury (Eyles *et*

al., 2004). However, impact of tannins on soil fungal community may be difficult to conclude. Tannins did not negatively affect on soil fungal populations (Baptist *et al.,* 2008) but high tannin concentration, which produced by some plants, can inhibit fungal growth (Nichols-orians, 1991). In this study, tannins may influence soil fungi and thus discrimination of fungal community between healthy trees and TPN affected trees was observed. Some fungi in soil can degrade tannins and can adjust itself to high concentration of phenol (Makkar *et al.,* 1993; Gamble *et al.,* 1996; Bhat *et al.,* 1997; Pinto *et al.,* 2001; Mutabaruka *et al.,* 2007). In this study, some fungi in soil have ability in enzymatic production for degrading tannins such as fungal species in genus of *Paecilomyces* (Mahendran *et al.,* 2006; Battestin and Macedo, 2007; Guedegbe *et al.,* 2008) and *Trichoderma* (Bajpai and Patil, 1996; 1997). These fungal genera existed dominantly in soil with TPN affected tree. Furthermore, *Paecilomyces, Trichoderma, Thielavia* and *Corynascus* also have ability in degrading plant residues (Eslyn *et al.,* 1975; Gerber *et al.,* 1997; Maheshwari *et al.,* 2000; Kluczek-Turpeinen *et al.,* 2003; Ryckeboer *et al.,* 2003; Martínez *et al.,* 2005; Lopez *et al.,* 2006).

This study suggested that the TPN has influenced soil fungal community. Similar dendrogram of 28S rDNA-DGGE revealed that fungal cluster under TPN affected trees (BH) was discriminated with under healthy trees (HH). The secondary metabolites and root exudation of TPN affected trees may influence fungal diversity.

III.1.8 CONCLUSION

The soil biological activities in *H. brasiliensis* plantation seem to be affected by TPN in rainy season because of climate condition. Soil biological activity analysis carried out in this study showed for the first time that some biological activity indicators can help to distinguish between healthy trees (H and HH) and trees affected by TPN (B and BH). This distinguish seems to be useful for observing the impact of TPN on the ecology of *Hevea brasiliensis* plantations. The principal component analysis showed that High cellulase, xylanase and amylase activities were associated with healthy trees and were lower near diseased trees. PCA also indicated that N-acetyl-glucosaminidase activity was associated with the zone between healthy and affected trees (BH). In addition, macrofaunal investigation revealed that the termite density was strongly associated with TPN. Soil microbial analysis showed that *Paecilomyces lilacinus* and *Trichoderma asperellum* were abundant in soil with TPN. Furthermore, soil microbial community structure was investigated using 28S DNA-DGGE. The similar dendrogram revealed that fungal cluster of TPN affected trees (B and

BH) was clearly separated from fungal cluster of healthy trees (H and HH). It would seem that polysaccharides activities, N-acetyl-glucosaminidase activity, termite density, and *Paecilomyces lilacinus* and *Trichoderma asperellum* abundance can be used as an indicator of the impact of TPN on soil ecology. However, the production of secondary metabolites as a result of TPN must be investigated.

CHAPTER III.2
IMPACT OF SOIL ORGANIC AMENDMENTS ON SOIL BIOLOGICAL ACTIVITIES

Abstract

Soil quality is necessary to sustain *Hevea brasiliensis* productivity. Soil amendment has been known to improve soil fertility. In this study, biological activites in soil with different amendments including no organic input, external organic input and *Pueraria* cover crop + external organic input were investigated. The biological and soil chemical parameters measured can separate soils according to their management. Most enzymes measured were high in amended soils which had high organic carbon and organic matter. Principal components analysis revealed that high xylanase and cellulase activities were associated with soil under *Pueraria* cover crop + external organic input. The highest FDA hydrolysis was the highest in soil with external organic input probably because of commercial compost added to soil. Investigating macrofauna community showed that total macrofauna abundance was the highest in plantations with organic amendment. Ants, termites, Arachnida and Diplopoda densities were higher in plantations with organic amendments than in plantation without organic amendment. Soil organic carbon and organic matter were higher in amended soils than in unamended soil and had the highest in soil with *Pueraria* cover crop + external organic input. The results show that plantation with *Pueraria* cover crop + external organic input had high enzyme activities, macrofauna density and soil chemistry. This plantation, *Hevea* tree had also the highest growth and latex yield. Thus, this study suggests that *Pueraria* cover crop + external organic input seem to be proper soil management for *H. brasiliensis.*

III.2.1 INTRODUCTION

Hevea brasiliensis or para rubber tree is an economically important tree in Thailand because its latex can increase public and rural revenue. The natural rubber from *H. brasiliensis* is used as component of many products, especially, transportation such as tires and tire products. The *H. brasiliensis* was the first established in south of Thailand and nowadays is extended to northeast. However, many areas in northeast Thailand have the problem in low soil fertility because of soil characteristic. The soils in this region have the most proportion of sand, which is the low capacity to hold mineral nutrients and moisture existing in soil. This can affect plant productivity in both quantitative and qualitative. The *H. brasiliensis* is a humid tropical tree and good soil fertility significantly affects latex yields (Akpan *et al.,* 2007). Therefore, agricultural practices that enhance soil quality are needed particularly for this region. Improvement of soil quality has the aim in enhancing physical, chemical and biological properties of soil (Lavelle, 1988; Bandick and Dick, 1999; Lado *et al.,* 2004). Enhancing these soil properties can lead to sustainable agriculture by increasing crop productivity and maintaining long-term soil fertility.

Organic amendment can improve soil quality and sustain crop productivity by increasing organic matter. Organic manures are acknowledged that it can use to solve the adverse effects on ecosystem because it can modify the properties of soil to be favorable on living organisms in ecosystem. Soil organic amendment using external organic materials and green manure/cover crop are generally practiced in agricultural system to enhance soil fertility. Using exogenous organic materials is increasing diversity of organic resources and consequently, the biological diversities are increased. Studies have been suggested that organic manure can increase soil organic matter and provide long-term soil productivity (Chander *et al.,* 1997a; Chander *et al.,* 1998). It has been known that the organic matter in soil is important source of energy for living organisms in soil. For instance, the diversity and quantity of organic matter in soil can increase diversity and abundance of soil macrofauna. Under the cover cropping system, the density of cover plant roots increase soil pore sizes and enhance soil environment to suite for soil macrofauna (Carof *et al.,* 2007). The macrofauna is useful to soil fertility and soil structure because its activities play role in soil nutrient cycling, which result in increasing soil fertility (Lobry de Bruyn and Conacher, 1990; Lavelle *et al.,* 1993; Mora *et al.,* 2005) Additionally, the macrofauna activity can enhance soil structure including soil porosity and soil aggregate stability (Lobry de Bruyn and Conacher, 1990; Mando *et al.,* 1996; Mando, 1997; Lavelle, 1997a; Pitkänen and Nuutinen, 1998;

VandenBygaart *et al.,* 2000). The good soil aeration and water infiltration contribute to plant root development and crop productivity is consequence. Furthermore, microbial activities were stimulated by soil macrofauna (Scheu, 1987; 1990; Binet *et al.,* 1998; Holt, 1998; Daube and Wolters, 2000; Scullion and Malik, 2000; Chaou *et al.,* 2003; Desjadins *et al.,* 2003; Ndiaye *et al.,* 2004; Stadler *et al.,* 2006). Some invertebrates in soil associate with soil microorganisms. For instance, the higher nitrifying bacteria were detected in earthworm burrows (Parkin and Berry, 1999). Additionally, some microbial decomposers, which can degrade cellulose and lignin, inhabit symbiotically in guts of earthworm and termites (Rouland *et al.,* 1988; Rouland *et al.,* 1991; Zhang *et al.,* 1993; Rouland-Lefevre *et al.,* 2002; Hyodo *et al.,* 2003). Organic amendments not only enhance soil quality but also decrease plant pest levels such as nematodes and weeds (Isaac *et al.,* 2007).

Organic matter in soil can increase diversity and activity of soil microorganisms. However, quality of organic materials is an important to enhance soil microbial biomass. The microbial activity contributes to soil fertility (Wander *et al.,* 1994; Nelson and Mele, 2006; Fliessbach *et al.,* 2007; Hamer *et al.,* 2008) because it can decompose organic matter to available forms for plants. The soil microbial activity was often measured using values of enzymes in soil. Soil enzyme activity is an important to sustain fertility of soil because it plays role in degradation of organic materials. The organic decomposition greatly enhances available nutrients and cycling of nutrients, which benefit to plant productivity (Dick *et al.,* 1988; Bandick and Dick, 1999; Hu and Cao, 2007). However, values of enzymes in soil depend on quality and type of organic amendment because soil practices directly affect soil biomass and activities (Wander *et al.,* 1994; Stark *et al.,* 2007; Stark *et al.,* 2008; Liu *et al.,* 2009). Enzyme activities have important roles in biochemical functioning of soil including decomposition of soil organic matter and nutrient cycling (Gianfreda *et al.,* 2005; Acosta-Martínez *et al.,* 2007; Chen *et al.,* 2008; Sardans *et al.,* 2008). Additionally, the potential of soil degradation can be detected by information of soil enzyme activities (Trásar-Cepeda *et al.,* 2000). The measurement of integrative activity of several soil enzymes is greatly efficient to predict the quality of soil because soil enzyme expression is closely related to soil physical and chemical properties, and soil microbial community (Decker *et al.,* 1999; Andersson *et al.,* 2004; Roldán *et al.,* 2005; Acosta-Martínez *et al.,* 2007; Iovieno *et al.,* 2009). Further, change in soil quality can be predicted by soil enzymes before it is detected by other soil analyses (Ndiaye *et al.,* 2000). Soil enzyme activity is nowadays used to monitor the quality of soil under different management because it is sensitive to change in soil environment (Acosta-Martínez and Tabatabai, 2000; Acosta-Martínez *et al.,* 2007). In addition, soil enzyme

activity can be used as sensitive index for soil biological stress (Garcia and Hermandez, 1997). Therefore, it is useful as early indicators of biological changes (Bandick and Dick, 1999). Examination of enzyme activities affected by soil organic amendments can provide understanding the progress of ecological interaction between soil enzymes and physiochemical properties. This knowledge can be applied for soil fertility development for *Hevea* plantation.

The objectives of this study were: i) to evaluate the impact of different organic amendments on soil macrofauna community and soil enzyme activities in *Hevea* plantation, and ii) to examine interaction between soil biological activities and soil physiochemical properties in different soil organic amendments. Soil biological activities greatly influence sustainable agriculture because it significantly associates with soil fertility. Information of soil organic amendments of *Hevea* plantation is important to long-term maintenance of soil quality and to sustain latex production.

III.2.2 EXPERIMENTAL DESIGN

Three *Hevea* plantations were selected by depending different soil managements as described in table 7**.** Samples were taken during the rainy season (July to October 2006) and dry season (December 2008). In plantation without organic amendment (S1), 10 sampling zones under midway between trees were selected randomly. In plantation with organic amendment (S2 and S3), each plantation, the 40 sampling zones under midway between trees were selected. The experimental design is an ordered block consisting of 20 x 20 meters.

Table 7 Description of three experimental sites with different soil managements

	S1	S2	S3
Date of planting	1997	1998	1999
Hevea varieties	RRIM 600	RRIT 251	RRIT 251
Before plantation established	Sugarcane	Sugarcane	Sugarcane
Soil managements			
- Chemical fertilizers	22-78-0 (624 kg/ha)	15-7-18 (357 kg/ha)	15-7-18 (357 kg/ha)
- Organic amendment			2000-2002 Cover cropping with *Pueraria phaseoloides* (6.25 kg of dry seed/ha)
		2003 eucalyptus bark residue (62.5 t/ha)	2003 eucalyptus bark residue (62.5 t/ha)
		2003 sugarcane filter cake (waste from sugar production) (12.5 t/ha)	2003 sugarcane filter cake (waste from sugar production) (12.5 t/ha)
		2003 Commercial compost (made from eucalyptus residue) (223.19 kg/ha)	
Latex yield (kg/ha/day)	13.75	26.9	33.1

III.2.3 IMPACT OF SOIL AMENDMENTS ON PLANT GROWTH

The averages of perimeter of *Hevea* tree were 46.8, 45.1 and 47.2 cm under plantation without organic input (S1), with external organic input (S2) and with *Pueraria* cover crop + organic input (S3), respectively (Figure 7). The *Hevea* growth in plantation with *Pueraria* cover crop + organic input (S3) was significantly higher (P>0.05) than in plantation with external organic input (S2).

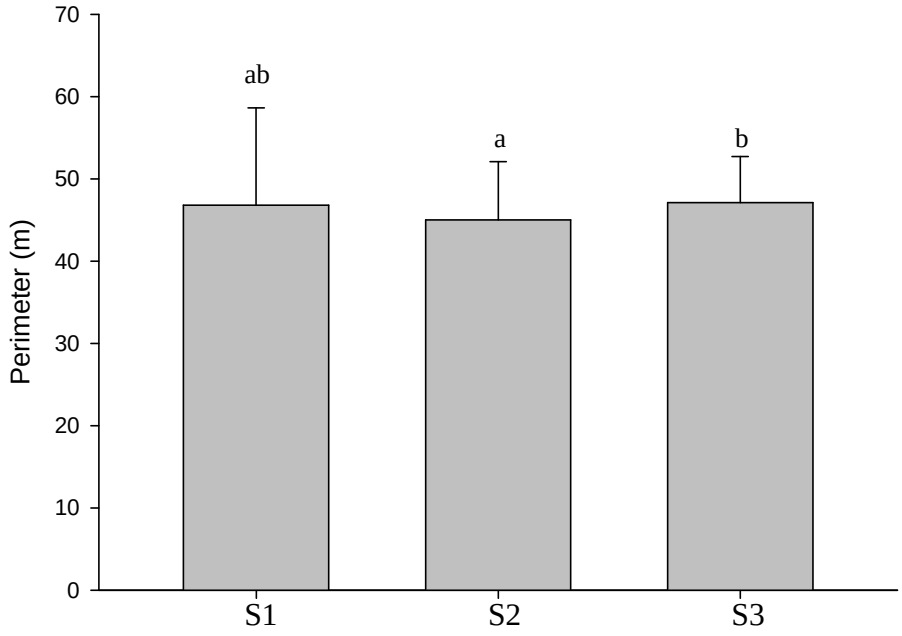


Figure 7 *Hevea* growth under different soil amendments; without organic amendment (S1), with external organic input (S2) and with *Pueraria* cover crop + organic input (S3). Means and standard deviation (n=40 for S1 and n=140 for S2 and S3); bars with the same letter are not significantly different at P<0.05

III.2.4 IMPACT OF SOIL ORGANIC AMENDMENTS ON SOIL PROPERTIES

Soil properties in rainy and dry seasons were showed in table 8.

Soil pH

The assay of soil pH showed that the soils were more acidic in rainy season than in dry season. In each season, no significant difference was observed for soil with no organic amendment (S1), organic input (S2) and *Pueraria* cover crop + organic input (S3).

Soil organic carbon

In each plantation, organic carbon in soil was higher in rainy season than in dry season. In both seasons, organic carbon in soil with *Pueraria* cover crop + external organic input (S3) was the highest and soil without external organic input (S1) was the lowest.

Total nitrogen

For total nitrogen, there was no significant difference between the seasons or the amendments.

C:N ratio

In each plantation, soil C:N ratio was higher in rainy season than in dry season. In both seasons, soil C:N ratio was the highest in plantation with *Pueraria* cover crop + organic input (S3) and was the lowest in plantation without external organic input (S1).

Soil organic matter

In each plantation, soil organic matter was higher in rainy season than in dry season. In both seasons, soil organic matter was the highest in plantation with *Pueraria* cover crop + organic input (S3) and was the lowest in plantation with no organic amendment (S1).

Cation exchange capacity (CEC)

In both seasons, the cation exchange capacity was the highest in soil with *Pueraria* cover crop + organic input (S3). The cation exchange capacity of soil was not significantly influenced by tested soil amendments or by the season.

Soil moisture

In rainy season, the soil with no organic amendment (S1) had the highest in moisture and was significantly higher ($P<0.01$) than soil with *Pueraria* cover crop + organic input (S3) and with organic input (S2).

Clay content

In rainy season, the clay content was the highest in soil with no organic amendment (S1) and was significantly higher ($P<0.01$) than soil with *Pueraria* cover crop + organic input (S3). There was no significant difference in clay content for samples taken from soil with organic amendments (S2 and S3)

In dry season, the soil clay contents in various plantations were not significantly different.

Table 8 Soil characteristics in three different soil amendments; no organic amendment (S1), amended with external organic input (S2), amended with *Pueraria* cover crop + external organic input (S3) during rainy and dry season

	S1	**S2**	**S3**
Rainy season			
pH	4.50 (0.15) a	4.45(0.26) a	4.55 (0.13) a
Organic carbon (%)	0.78 (0.08) a	1.04 (0.27) a	1.50 (0.56) a
Total nitrogen (%)	0.08 (0.01) ab	0.08 (0.01) a	0.09 (0.00) bc
C/N ratio	9.94 (0.54) a	14.03 (4.53) a	17.22 (6.52) a
Organic matter (%)	1.34 (0.14) a	1.79 (0.47) a	2.58 (0.97) a
Cation exchange capacity (%)	4.43 (0.20) a	4.36 (0.54) a	5.09 (0.96) a
Moisture (%)	16.22 (0.98) a	9.60 (0.79) c	11.63 (1.05) b
Clay (%)	9.54 (0.68) bc	8.65(0.35) ab	7.66(0.62) a
Dry season			
pH	5.02 (0.14) [a]	5.41 (0.28) [a]	5.29 (0.31) [a]
Organic carbon (%)	0.71 (0.13) [a]	0.82 (0.08) [a]	0.90 (0.27) [a]
Total nitrogen (%)	0.11 (0.01) [a]	0.10 (0.02) [a]	0.09 (0.01) [a]
C/N ratio	7.68 (0.99) [a]	8.87 (3.15) [a]	10.63 (2.68) [a]
Organic matter (%)	1.23 (0.22) [a]	1.41 (0.13) [a]	1.55 (0.47) [a]
Cation exchange capacity (%)	5.20 (0.08) [a]	6.82 (0.85) [a]	7.08 (1.39) [a]
Moisture (%)	3.99 (0.58) [a]	4.04 (1.38) [a]	2.30 (0.59) [a]
Clay (%)	9.97 (1.11) [a]	8.12 (1.09) [a]	8.37 (0.55) [a]
n	3	5	4

[a] Mean within a column followed by same lower case letter are not significantly different at $P<0.05$ using Tukey HSD. Numbers in parentheses indicate standard deviation.

Principal Component Analysis

Principal component analysis on soil variables in rainy and dry seasons was showed in figure 8. In rainy season, PCA showed axis 1 and axis 2 for 53.8% and 17.3% of the variance, respectively. In dry season, PCA showed axis 1 and axis 2 for 50.5% and 24.5% of the variance, respectively. Testing the significance of groupings (sites) was carried out on 10,000 permutations and was significantly discriminated ($P<0.002$) in both seasons.

Principal component analysis revealed that, both seasons, soil with no organic amendment (S1) was clearly separated from the amended soils (S2 and S3) by the highest soil clay content and moisture. In the dry season, the highest total N was associated with soil under no organic amendment (S1). Furthermore, the highest values of C:N ratio, organic matter and organic carbon were associated with soil under *Pueraria* cover crop + external organic input (S3), particularly in rainy season. High values of cation exchange capacity (CEC) in soil were associated with plantations with organic amendments (S3 and S2 for the rainy and dry seasons, respectively).

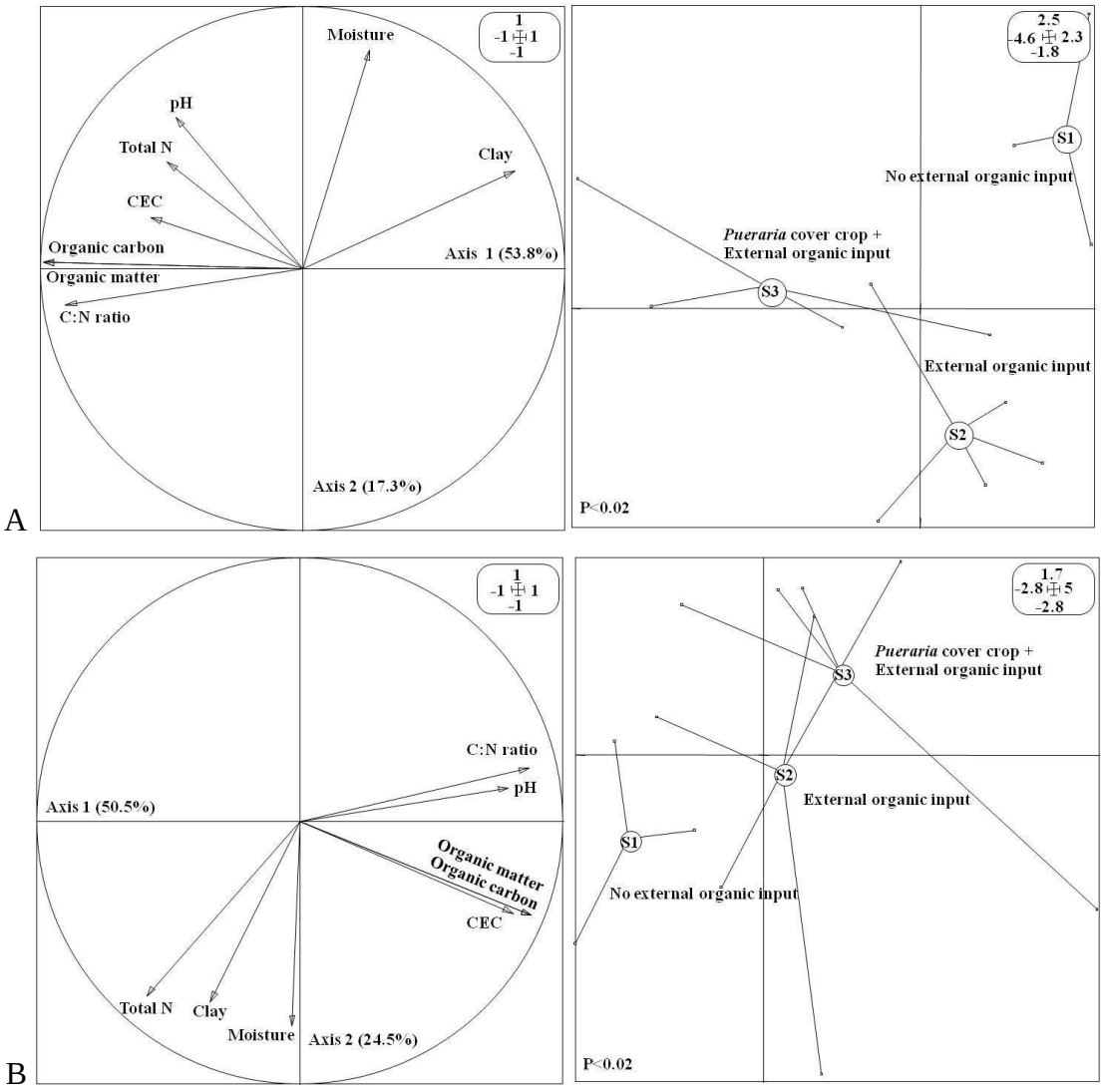


Figure 8 Principal component analysis showed the relationship between soil variables and organic managements during rainy (A) and dry (B) seasons

III.2.5 IMPACT OF SOIL ORGANIC AMENDMENTS ON MACROFAUNA COMMUNITY

III.2.5.1 Macrofauna density

For each *Hevea* plantation, macrofauna abundance in soil was higher than in litter, except macrofauna abundance in plantation without organic amendment (S1) in dry season where macrofauna cannot be detected (Figure 9). The highest density in soils was due to the large numbers of termites and ants.

The determination of macrofauna in litter showed that in each plantation, ant group was the largest taxonomic unit, except for S1 in dry season where “others” (particularly hemiptera) is the most abundant group. Except this plot, the litter macrofauna was not greatly different: ants, Arachnida and termites densities were the highest in plantation independently of the season.

The investigation of soil macrofauna showed that macrofauna in plantations with organic amendment (S2 and S3) was higher abundant than in plantation without organic amendment (S1). Moreover, no macrofauna population in plantation without organic amendment (S1) was observed in dry season. Each plantation, proportions of termite, ant and Coleoptera were greater than others. Earthworms were collected only in rainy season and the most abundant was in plantation without organic amendment (S1).

The analysis of macrofauna by combining results between litter and soil macrofauna revealed that the macrofauna density in plantations with organic amendment (S2 and S3) was higher than in plantation without organic amendment (S1), the highest density was in plantation with *Pueraria* cover crop + external organic input (S3) in rainy season. For S2 and S3 plantation, termites, ants, Coleoptera and Arachnida were the largest taxonomic groups. Coleoptera, earthworm and “others” populations were the most present in plantation without organic amendment (S1).

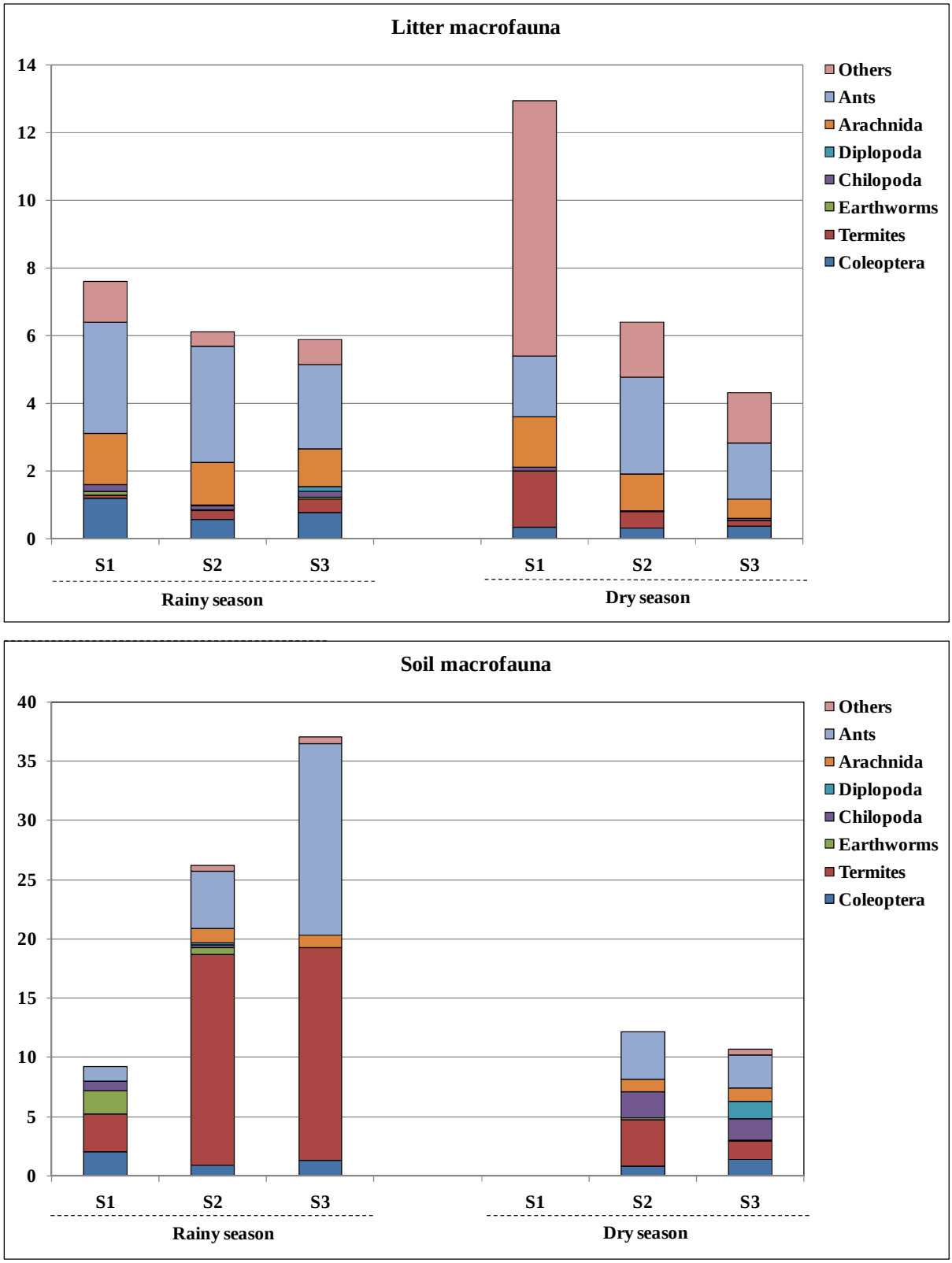


Figure 9 Macrofauna densities (individual m^{-2}) in litter and soil under plantation with no organic input (S1), with external organic input (S2) and with *Pueraria* cover crop + external organic input (S3)

III.2.5.2 Macrofauna diversity

Macrofauna diversity in each plantation was investigated by combining results of litter and soil macrofauna obtained during the two seasons (Table 9). Plantations with organic amendment (S2 and S3) were the highest macrofauna diversity.

Coleoptera, termites and ants were the most abundant groups and found in both litter and soil, these were studied separately. The level of genus, possible, to species was determined. The Coleoptera diversity showed that six genera were identified in plantation without organic amendment (S1) and with organic amendment (S2), seven in S3. The genus *Cerambycidae* was not found in S1 and S2 than *Dytiscidae* was not observed in plantation with external organic input (S2). For termite, two genera (*Ancistrotermes* and *Macrotermes*) were observed in all plantations.

Concerning ant diversity, 11 species were identified in plantation without organic amendment (S1), 18 in plantation with external organic input (S2), 16 in plantation with *Pueraria* cover crop + external organic input (S3). The *Odontoponera transversa*, *Paratrechina longicornis*, *Anoploepis gracilipes*, *Oecophylla longinoda*, *Camponotus* sp 1, *Camponotus* sp 3, *Camponotus* sp 5 and *Pachycondyla sp.* were found in all plantations. *Hypoponera* sp 1, *Hypoponera* sp 2, *Odontomachus* sp 1 and *Camponotus* sp 2 were detected in plantations with organic amendment (S2 and S3). The *Morphospecies* sp 2 was only found in plantation without organic amendment (S1). *Ectatomma*, *Odontomachus* sp 2, *Odontomachus* sp 3, *Camponotus* sp 4 and *Camponotus* sp 6 were only detected in plantation with external organic input (S2). *Pheidole* sp 1, *Morphospecies* sp 1 and *Morphospecies* sp 3 were only found in plantation with *Pueraria* cover crop + external organic input (S3).

Table 9 Diversity of macrofauna under different soil organic managements; no organic input (S1), external organic input (S2) and *Pueraria* cover crop + external organic input (S3)

	Litter						**Soil**					
	Rainy			**Dry**			**Rainy**			**Dry**		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Coleoptera												
Carabidae sp.	*		*	*	*	*			*		*	*
Cerambycidae sp.						*						
Coccinellidae sp.					*						*	
Elateridae sp.	*	*				*						
Scarabaeidae sp.	*	*	*			*	*					*
Staphylinidae sp.	*			*				*	*		*	
Tenebrionidae sp.	*	*	*	*	*	*	*	*	*		*	*
Dytiscidae sp.	*					*						
Larva	*	*	*				*	*	*		*	
Termites												
Ancistrotermes sp.						*	*	*	*			
Macrotermes sp.	*	*	*	*	*	*	*	*	*		*	*
Ants												
Odontoponera transversa	*	*	*					*	*			
Hypoponera sp. 1		*	*					*				
Hypoponera sp. 2		*	*					*	*			
Ectatomma sp.		*										
Odontomachus sp. 1		*	*		*	*		*				
Odontomachus sp. 2		*										
Odontomachus sp. 3								*				
Pachycondyla sp.				*	*	*					*	*
Platythyrea sp.				*								
Pheidole sp. 1			*									
Morphospecies sp. 1									*			
Morphospecies sp. 2							*					
Morphospecies sp. 3			*									
Calyptomyrmex sp.						*					*	*
Paratrechina longicornis	*	*	*						*			
Anoploepis gracilipes	*	*	*		*	*		*	*		*	*
Oecophylla longinoda	*	*	*		*	*						
Camponotus sp. 1	*	*	*		*	*		*			*	*
Camponotus sp. 2		*	*	*	*	*					*	*
Camponotus sp. 3	*	*	*	*	*	*		*			*	*
Camponotus sp. 4		*										
Camponotus sp. 5	*	*	*									
Camponotus sp. 6		*										
Larva		*	*	*	*	*	*	*	*			

Diversity Index

The specific data obtained on the major group were used to calculate several diversity indexes: specific richness, Shannon index and diversity Simpson index (Table 10).

The specific richness, which considers only the number of species or morphospecies, was higher in plantation with soil organic managements (S2 and S3) than in plantation without organic management (S1).

Shannon and Simpson indexes indicated that the repartition is more homogenous in S1 and S3 than in S2, which the macrofauna community was dominated by few species.

Moreover, this investigation notice that the species composition of the macrofauna is closer between S2 and S3 (55% common species) than between S1 and the two other plots (39 % for S2 and 43% for S3 respectively).

Table 10 Specific richness, Shannon index and Simpson index of macrofauna in *Hevea* plantation without external organic input (S1), with external organic input (S2) and with *Pueraria* cover crop + external organic input (S3)

	Specific richness	**Shannon**	**Simpson**
S1	19	1,10a	0,98a
S2	26	0,82b	0,67b
S3	25	0,98ab	0,95a

III.2.6 IMPACT OF SOIL ORGANIC AMENDMENTS ON SOIL ENZYME ACTIVITIES

The expression of soil enzyme activities from samples in rainy and dry seasons was showed in table 11.

Polysaccharidases

Cellulase activity is not significantly different between the different plots nether in relation to the season. Xylanase activity is higher during rainy season and is in soils with organic amendment (S2 and S3). The amylase activity is also higher in rainy season but activity was not significantly different in soils without organic amendment (S1).

Heterosidases

The β-glucosidase activity in soil without organic amendment (S1) was significantly higher ($P<0.05$) than in soils with organic amendments (S2 and S3) in rainy and dry season.

N-acetyl-glucosaminidase activity is higher during the dry season but it was not significantly between the plots.

Urease

Urease activity was higher in dry season than in rainy period for all the plots. Plantation with external organic input (S2) had the highest urease activity and it was significantly higher ($P<0.05$) than plantations with *Pueraria* cover crop + organic input (S3) and without organic amendment (S1). In rainy season, urease activity was highest in soil without amendment (S1) and was significantly higher ($P<0.05$) than in soil with *Pueraria* cover crop + external organic input (S3).

Fluorescein diacetate (FDA) hydrolysis

In each plantation, FDA hydrolysis activity was higher in rainy season than in dry season. However, the significantly higher difference ($P<0.05$) was observed only in plantation without external organic input (S1). In each plantation, FDA hydrolysis activity was not significantly different among plantations.

Table 11 Soil enzyme activities (μg of product g^{-1} soil h^{-1}) in three different organic amendments; no organic amendment (S1), amended with external organic input (S2), amended with *Pueraria* cover crop + external organic input (S3) during dry and rainy periods.

	S1		S2		S3	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
Cellulase	1.52 (0.33) [a]	1.76 (0.53) [a]	3.38 (1.42) [a]	3.27 (1.06) [a]	3.30 (1.46) [a]	2.91 (0.85) [a]
Xylanase	3.79 (0.55) [b]	0.75 (0.79) [c]	5.08 (1.73) [a]	3.51 (1.03) [b]	7.38 (3.88) [a]	4.28 (1.92) [ab]
Amylase	12.90 (1.13)[a]	5.57 (0.56) [c]	10.14 (2.61) [ab]	7.21 (1.02) [bc]	13.83 (2.40) [a]	3.92 (2.04) [c]
β-glucosidase	32.15 (10.88) [a]	33.97 (8.05) [a]	12.33 (4.52) [b]	28.83 (3.19) [ab]	8.21 (2.00) [b]	15.69 (2.43) [b]
N-acetyl-glucosaminidase	1.25 (0.17) [b]	7.18 (0.54) [ab]	0.84 (0.56) [b]	9.09 (2.18) [a]	1.46 (0.43) [b]	5.73 (1.55) [ab]
Urease	1.70 (0.29) [b]	3.17 (0.13) [a]	1.31 (0.20) [bc]	5.51 (1.23) [a]	1.00 (0.26) [c]	2.42 (0.52) [ab]
FDA hydrolysis	5.30 (1.22) [a]	1.17 (0.14) [b]	6.63 (1.69) [a]	3.19 (0.78) [ab]	4.79 (1.07) [ab]	2.46 (0.54) [b]

[a] Mean within a row followed by same lower case letter are not significantly different at $P<0.05$ using Tukey HSD. Numbers in parentheses indicate standard deviation.

III.2.7 DISCUSSION

III.2.7.1 Soil quality and plant productivity

Soil quality is generally used to evaluate sustainable land management in agroecosystems (Carter, 2002). Soil organic matter is an important indicator for agricultural soil (Lal and Kimble, 1997). Soil organic matter is efficient storage for available nutrients (such as C, N, P and S) and it can improve soil properties, soil fertility and crop production. Thus, improving soil organic matter can improve soil quality (Reeves, 1997; Freixo *et al.,* 2002). However, soil organic matter is influenced by agricultural management (Liu *et al.,* 2006). In this study, the parameters for assessing soil quality including organic carbon, C:N ratio and organic matter were higher in soils with organic amendments (S2 and S3) than in soil without organic amendment (S1). This indicated that unamended soil (S1) had the lowest soil quality. The lower carbon and organic matter contents in unamended soil (S1) may due to lower organic materials in soil (Golchin *et al.,* 1995). In contrast, addition of organic materials to soil can increase the level of soil organic matter (Carter *et al.,* 1998). Furthermore, low residue inputs have caused rapid losses of soil organic matter (Lal and Kimble, 1997) while plant residue additions can increase soil carbon content (Bossuyt *et al.,* 2002). In this study, the quality of organic materials in both amended soil (S2 and S3) may be higher than in unamended soil (S1). It has documented that quality of organic residue can influence the quantity of soil organic matter (Juma, 1993).

Principal components analysis revealed that soil amended with *Pueraria* cover crop + organic input (S3) was associated with highest organic carbon and organic matter. Studies suggested that cover crops can increase organic carbon and nitrogen because amounts of organic residue in soil were increased (McVay *et al.,* 1989; Kuo *et al.,* 1997; Seo and Lee, 2008). It has been reported that the legume has high aboveground biomass and foliar N content (Agamuthu and Broughton, 1985). Cover crop also increases soil organic matter due to increased plant residue addition to soil (Sainju *et al.,* 2000).

However, using legume cover crop need long-time for improving soil quality. In this study, *Pueraria* cover crop was terminated when *Hevea* canopy was close (approximately 3 years). Studies have reported that the long-term *P. phaseoloides* (more than 10 years) cover crop significantly affected soil organic C and N. (Dinesh *et al.,* 1999; Dinesh *et al.,* 2004; Dinesh *et al.,* 2009). Wander et al. (1994) reported that the legume cover cropped soil with 10

year-period significantly increased in soil carbon and organic matter compared to conventional soil. It has demonstrated that continuous legume cover crop can improve soil organic matter and sustain crop yield (Sainju *et al.,* 2002; Seo and Lee, 2008). Hu et al. (1997) demonstrated that short-term cover crop affected transient soil microbial dynamics and nutrient availability. Several studies have documented that microbial biomass and nitrogen mineralization were rapidly increased in first few weeks after cover crop incorporation and then returns to pre-incorporation (Wyland *et al.,* 1996; Lundquist *et al.,* 1999; Jackson, 2000; Sung *et al.,* 2008).

Increasing soil organic matter and soil organic carbon have affect on soil properties and increasing available nutrients and consequently, crop yield is improved (Berzsenyi *et al.,* 2000; Onemli, 2004). In this study, the growth of *Hevea* tree was evaluated using perimeter estimation. The *Hevea* growth from plantation with *Pueraria* cover crop + organic input (S3) was significantly higher ($P<0.05$) than from plantation with organic input (S2). In addition, latex yield under plantation with *Pueraria* cover crop + organic input (S3) was the highest. It has been documented that the *P. phaseoloides* has ability in N-fixation (Vesterager *et al.,* 1995). In addition, legumes foliar generally have high abundance of other nutrients such as P, K and Mg (Franchini *et al.,* 2004; Njunie *et al.,* 2004; Armecin *et al.,* 2005). Therefore, the legume cover cropped soil contains the available nutrients for plant development. Studies have been reported that legume cover crop can increase crop yields (Abdul-Baki and Teasdale, 1993; Armecin *et al.,* 2005; Venkateswarlu *et al.,* 2007). Under plantation with *Pueraria* cover crop (S3) in this study, the *P. phaseoloides* was planted as cover crop in the interspaces of plantation in the early establishment of plantation. The trees in the early stage may be positively affected from ability of legume in N-fixation process and releasing nitrogen forms after its decomposition (Lundquist *et al.,* 1999; Jackson, 2000). It has demonstrated that cover crop can provide nitrogen source affecting plant growth at early stage (Sung *et al.,* 2008). Additionally, when the legume was terminated, it can release the nutrients by microbial decomposition. This can greatly contribute the growth of tree. The perfect development of trees in the early stage may have significant effect on long-term plant productivity. In this study, plantation with organic input (S2) was the older than plantation with *Pueraria* cover crop + organic input (S3) but *Hevea* growth rate and latex yield were lower. In addition, principal components analysis showed that most enzymes tested were high in soil under plantation with *Pueraria* cover crop (S3). This may supply nutrients available for *Hevea* productivity.

This study observed that plantation without organic amendment (S1) had the lowest latex yield. This may due to high incidence of trunk phloem necrosis (TPN) in this plantation. Trunk phloem necrosis is the main constraint of latex production (Nicole *et al.*, 1991; Nandris *et al.*, 2005). High incidence of TPN may due to coarse quality of soil. (Nandris *et al.*, 2004; 2005) concluded that TPN disease may cause by low soil fertility which increasing the risk of the syndrome.

This investigation suggests that organic amendment can increase soil quality and using *P. phaseoloides* cover crop in the early stage of *Hevea* plantation positively affect soil fertility and potentially increase tree growth and latex yield.

III.2.7.2 Impact of soil amendment on macrofauna community

The principal components analysis revealed that systems with organic amendments (S2 and S3) were clearly separated from system without organic amendment (S1) in both rainy and dry seasons (Figure 10). Most macroinvertebrates were abundance in plantations with organic amendments, this due probably to the quality of organic materials adding to system. This study observed that the total macrofauna density was the highest in rainy season. It had been reported that the increasing organic matter in rainy period positively influenced macrofauna community by given new opportunities in colonization, refuge and foraging (Callisto *et al.*, 2002). Furthermore, plantation with *Pueraria* cover crop + organic input (S3) had the highest macrofauna density. It has been documented that *Hevea* plantation with continuous *P. phaseoloides* cover crop soon restores suitable conditions for soil macrofauna (Gilot *et al.*, 1995). In this study, the highest of macrofauna density under plantation with *Pueraria* cover crop-organic input (S3) in rainy season including, especially, ant, termite, Diplopoda and Coleoptera may be an integral part to sustain the growth of *H. brasiliensis* by improving soil structure and removing organic materials into deeper soil.

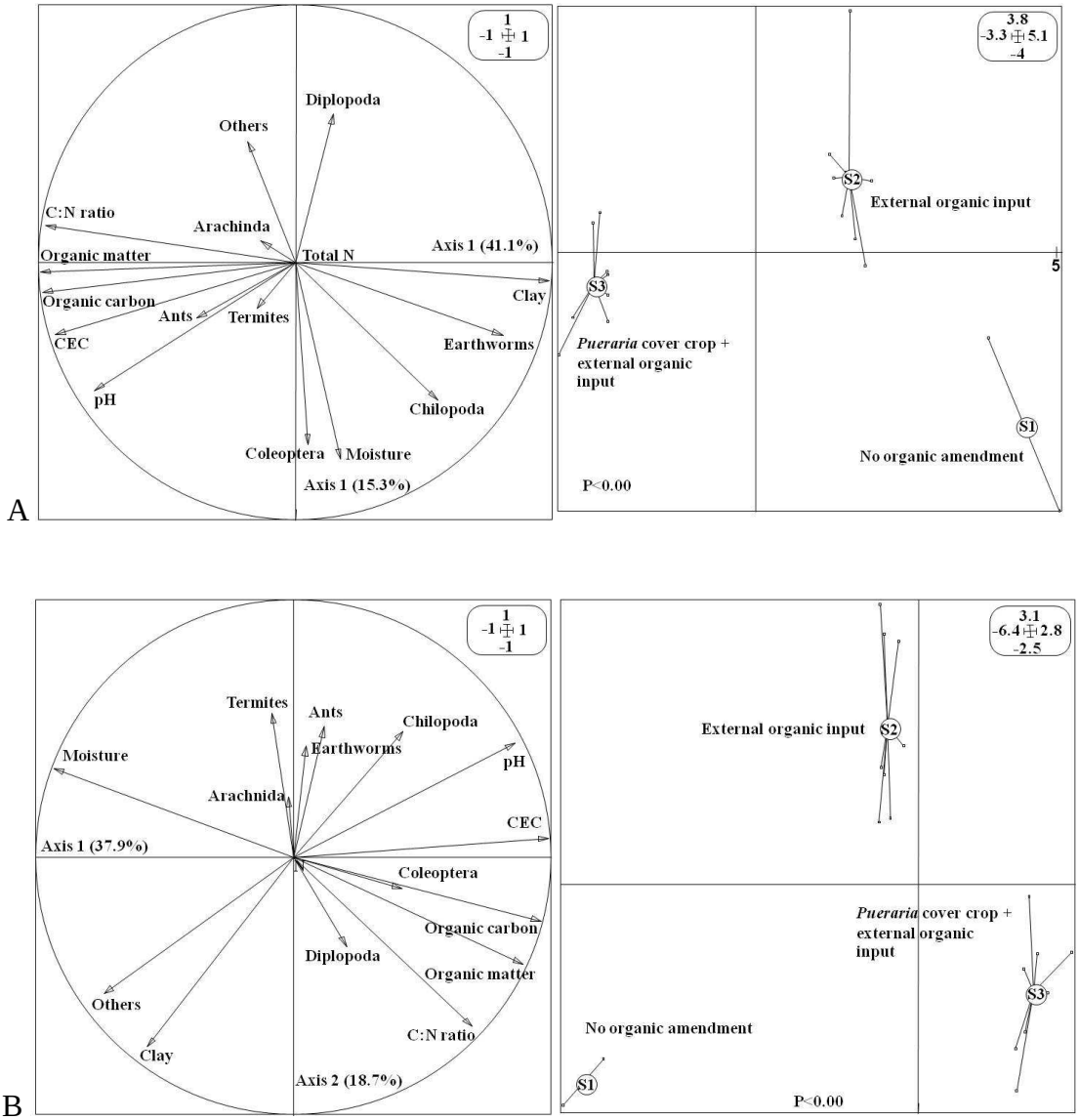


Figure 10 Principle component analysis on soil macrofauna and soil variables in rainy (A) and dry (B) seasons

Among macrofauna taxonomic groups in this study, ants and termites were mainly abundant organisms. Density of ants was higher in plantations with organic amendments (S2 and S3) than in plantation without organic amendment (S1) in both rainy and dry season. In addition, ant diversity was higher in plantations with organic amendments (S2 and S3) than in plantation without organic amendment (S1). These may result from the higher quantity and quality of organic matter present in these plantations. The abundance of ant is beneficial to soil structure because it can enhance soil porosity, aeration, infiltration and drainage (Lavelle and Spain, 2001b). This is important for plant root development and consequently, better

plant growth. Furthermore, ant density was the highest in plantation with *Pueraria* cover crop + organic input (S3) in rainy season. Laossi et al. (2008) suggested that some legume species significantly affected ant density but it did not influence density of other fauna group. This investigation found that diversity of ants under all plantations was decreased in dry period. However, some ants species including *Odontomachus* sp1. and *Camponotus* sp1. were only found in plantation with organic amendment (S2 and S3) in both rainy and dry season. It has been reported that, the number of species of ants depended on their ability in reproductive maintenance and adaptation on unfavorable environment (Bruyn, 1999). In addition, *Anoploepis gracilipes* and *Oecophylla longinoda* were found in all plantations in both rainy and dry season but they were not found in plantation without organic amendment (S1) in dry season. These species (*Odontomachus* sp1., *Camponotus* sp1. *Anoploepis gracilipes* and *Oecophylla longinoda*) may strongly associate with quality of organic amendments.

Termites were most abundance in soils with organic amendments (S2 and S3). Few species of termites had been observed. *Macrotermes* sp. was constantly associated with amended soils (S2 and S3). In addition, *Macrotermes* sp. was not found in soil without organic amendment (S1) in dry season. This indicated that quality of organic matter in soils with organic amendments (S2 and S3) may sustain this termite species. In addition, *Ancistrotermes* sp. was found in all soils in rainy season but it was not found in dry season. The decrease of soil moisture in dry season may negatively influence this species. However, the reason for termite distribution is not clear.

The rarity of earthworms and Coleoptera were found in this study. It has been reported that organic matter was necessary to keep an earthworm population (Gilot *et al.*, 1995). Earthworm community in soil is positively correlated with the quality of mulch cover (Edwards and Lofty, 1982). During rainy period, density of earthworm and Coleoptera were the lowest in plantations with organic amendments (S2 and S3). In addition, diversity of Coleoptera was the highest in plantation without organic amendment (S1). The eucalyptus bark, which is one of organic matter sources in amended plantations (S2 and S3) in this study, may be weakly palatable for macrofauna. High lignin and phenolic compounds contents of eucalyptus may be important factor in control of macrofauna population (Ganihar, 2003). Phenolic compounds, particularly, tannins are known to be defense mechanisms against herbivores (Coley and Barone, 1996; Ayres *et al.*, 1997; Nomura and Itioka, 2002). It has been shown that among tested tree plantations, eucalyptus plantation had the lowest in abundances and biomass of soil macrofauna (Warren and Zou, 2002). In addition, low palatability of *Hevea* litter may negatively affect occurrence of epigeic earthworm species

(Chaudhuri *et al.*, 2003). Several studies have reported that the quality of organic material significantly effects earthworm populations (Edwards and Lofty, 1982; Zou, 1993; Tian *et al.*, 1995; Gonzalez and Zou, 1999). Additionally, low earthworm population in plantations with organic amendments (S2 and S3) may have resulted from competition with termites (Gilot *et al.*, 1995). Furthermore, each experimental site in this study, soil was classified as sandy. (Mboukou-Kimbatsa *et al.*, 1998) found that the sandy soil had very few earthworms. In contrast, during dry period, the earthworm density was the lowest in plantation without organic amendment (S1) in dry season. (Lobry de Bruyn, 1997) suggested that the fallacious results on earthworm density due to sampling at inappropriate times. However, Lobry de Bruyn (1997) has been reviewed that earthworms were not convinced as indicators of soil sustainability because identical management practice can gave the resulting in different response to earthworms.

In this study, density and diversity of Coleoptera were varied change between seasons and between organic amendment systems. The distributions of Coleoptera are heterogeneous. Studies have been reported that Coleoptera abundance can alter between years (Holland *et al.*, 1998), within fields (Thomas *et al.*, 1998; Holland *et al.*, 1999) and between fields (Hance *et al.*, 1990).

The Diplopoda was presented in plantation with *Pueraria* cover crop + external organic input (S3) in both rainy and dry seasons while it was not found in plantation with external organic input (S2) in dry season and was not found in plantation without organic amendment (S1) in both rainy and dry seasons. It has been reported that the Diplopoda is probably associated with greater organic matter received from cover crop (Merlim *et al.*, 2005). Vohland and Schroth (1999) reported that *P. phaseoloides* litter had a favorable effect on Diplopoda abundance. Furthermore, it has documented that Diplopoda generally prefer soil with higher polyphenol (Lavelle and Spain, 2001a). The polyphenol containing in eucalyptus bark residues in this study may had effect on Diplopoda. For plantation with *Pueraria* cover crop + external organic input (S3), the large numbers of millipedes may be due to higher substrate palatability (Warren and Zou, 2002). Studies revealed that Diplopoda is an important factor to increase nutrient mineralization (Anderson *et al.*, 1983; Anderson *et al.*, 1985). This study observed that Diplopoda density under plantation *Pueraria* cover crop + external organic input (S3) was greatly lower in rainy season than in dry season. This probably is affected by ant and termite predators, which are higher abundance in rainy season.

Principal components analysis showed that Arachnida was the most abundance in plantations with organic amendments (S2 and S3). This group may be positively associated with quality of organic amendments. It has been reported that spider density was higher in organic farming system than in conventional farming system (Feber *et al.,* 1998). Spider community structure is depended on habitat differences (Bultman and Letz, 1982; Marc and Canard, 1997; Bonte *et al.,* 2002). Studies have been revealed that soil organic matter had a significant influence on some species of Arachnida population (Bardgett and Cook, 1998). Arachnida can contribute to decomposition and nutrient cycling by transporting fungal propagules (Heneghan *et al.,* 1999), comminuting organic matter (Seastedt, 1984) and mixing plant debris (Bardgett and Chan, 1999). In addition, spiders can be a good indicator group of ecological quality because they sensitive to ecological system such as vegetation structure and disturbance (Jocque *et al.,* 2005).

When both organic amendments (S2 and S3) are compared, individual densities of main macrofauna taxonomic groups were not greatly different between plantations with organic amendments (S2 and S3). Since environment is one of important factors controlling macrofauna community (Lavelle, 1997b; Nahmani *et al.,* 2006; Velasquez *et al.,* 2007; Yankelevich *et al.,* 2007), environmental condition in these plantations may be not outstandingly different. In addition, studies have been documented that macrofauna density is strongly affected by resource quantity (Vohland and Schroth, 1999; Laossi *et al.,* 2008). At the time of sampling, the amount of organic materials adding to both plantations (S2 and S3) may be similar.

Furthermore, macrofauna communities in this study were very change between rainy and dry seasons. For example, most macrofauna groups had the highest density under plantation with *Pueraria* cover crop-organic input (S3) in rainy period but they had the highest in plantation with organic input (S2) in dry season. Sroka and Finch (2006) found that droughty condition greatly affected macrofauna. It has been suggested that fallacious results can be obtained when macrofauna was sampled in improper period. (Lobry de Bruyn, 1997). However, the period for using *Pueraria* cover crop in this study may be insufficient to significantly increase macrofauna communities. It has been revealed that long-term legume fallows had positive impact on the diversity and functions of soil invertebrate (Sileshi and Mafongoya, 2006a).

In this study, macrofauna distributions were patchy. Soil property may be a cause of patchy distribution. It has been documented that low level of nutrients in sandy soils is a limiting factor for macrofauna (Mboukou-Kimbatsa *et al.,* 1998). The macrofauna cannot

readily respond to environmental change due to low fecundity, the interpretation of data were difficult because their distributions were patchy (Lobry de Bruyn, 1997). Additionally, the knowledge concerning effect of environmental factors on their distributions was not enough. It has been suggested that the measurement of macrofauna should be operated in short-term events (weeks to months) because macrofauna are active for short periods of time (Anderson, 1995).

Furthermore, macrofauna populations in this study were very low. It has been reported that most macrofauna populations in *Hevea* plantation were decreased after five years because the quality of litter due to ageing of the trees (Gilot *et al.*, 1995). However, chemical quality of organic materials (eucalyptus bark and sugarcane filter cake) needs to obtain for understanding the role of organic materials as substrate palatability.

III.2.7.3 Impact of soil organic amendment on soil enzyme activities

Numerous studies have been reported that enzyme activities are associated with increase in soil organic matter, organic C, total N content (Dick *et al.*, 1988; Chander *et al.*, 1997a; Pascual *et al.*, 1999; Acosta-Martínez *et al.*, 2007). Analysis of different enzyme activities together can provide information of the status of soil processes (Acosta-Martínez *et al.*, 2003). This investigation, activities of enzyme tested were generally higher in soils with organic amendments (S2 or S3) than in soil without organic amendment (S1). The higher activities in the soils with organic amendments (S2 or S3) may be due to addition of organic materials. The quantity and quality of organic substrates influence soil microbial processes, which are the contributing factor in increase of soil enzyme activities (Chander *et al.*, 1997b; Chander *et al.*, 1998).

Enzyme activities were strongly associated with microbial organisms, which are the main source of enzymes in soil and act important role in organic decomposition (Barbhuiya *et al.*, 2004; Ingram *et al.*, 2005; Devi and Yadava, 2006; Faterrigo *et al.*, 2006; Fosu *et al.*, 2007; Lucas *et al.*, 2007). Since expression of enzymes is microbial processes, the quantity of enzyme can generally indicate the levels of soil microbial population. The FDA ($3^{/}$,$6^{/}$ - diacetylfluorescein) is hydrolyzed by different enzymes such as proteases, lipases and esterases. The ability to hydrolyze FDA can provide correlation information on amounts of microbial decomposers and activities (Brunius, 1980; Ingham and Klein, 1982; Schnürer and Rosswall, 1982; Gaspar *et al.*, 2001). Numerous studies revealed that higher soil microbial activity caused by addition of exogenous source of organic matter (Saffigna *et al.*, 1989;

García-Gil *et al.*, 2000; Nilsson *et al.*, 2005; Stark *et al.*, 2007). Principal component analysis showed that the highest FDA hydrolysis activity was associated with amended soil with external organic input (S2). This indicated that population of microorganisms was the most in soil with external organic input (S2). The highest population may due to addition of commercial organic compost to soil. This compost may have large numbers of microorganisms. The FDA hydrolysis activity was decreased in dry season, probably, because this season is unfavorable for stimulation of microbial processes. However, this enzyme activity was significantly higher ($P<0.05$) in soils with organic amendments (S2 and S3) than in unamended soil (S1). This indicated that the higher quality of organic matter may associate with amended soil (S2 and S3) because it can protect loss of microbial processes.

Cellulose is an important carbon source for microorganisms in agricultural soil and it is degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulase. In addition, hemicellulose is a component of plant cell wall and it is degraded into glucose by xylanase, as the important carbon source. Principal component analysis revealed that high cellulase and xylanase activities were associated with soils under organic amendments (S2 and S3). High activities may due to accumulate the cellulolytic and xylanolytic materials in soil matrix, which derived from adding exogenous organic residues. Furthermore, xylanase activity was consistently the highest under soil with *Pueraria* cover crop+ organic input (S3) in both rainy and dry seasons. This result may due to *Pueraria* cover crop as the organic material. It has demonstrated that legume residue can increase soil xylanase activity (Rodíguez-Kábana, 1982).

The β-glucosidase is predominant in soil and it play important role in providing carbon source for microbes in soil. This enzyme hydrolyses cellobiose and various β-glucosides present in plant materials (Martinez and Tabatabai, 1997). This investigation showed that soil without organic amendment (S1) had the highest β-glucosidase activity. This result may due to decomposition rate of organic matter, which may be higher in soils with organic amendments (S2 and S3). The fast decomposition in organic residues may have lead to a decrease in available substrate for the β-glucosidase activity (Kourtev *et al.*, 2002). Likewise, this study found that β-glucosidase activity was increased in dry season. It has been reported that β-glucosidase is very sensitive to change in pH (Acosta-Martínez and Tabatabai, 2000). Increasing pH in dry season may result in increasing β-glucosidase activity in this study. Since β-glucosidase is sensitive to pH change, it can be used as biochemical indicator for determining changes in ecology causing from soil acidification.

The α-amylase is produced by plants, animals and microorganisms. This enzyme hydrolyzes starch to glucose which is the carbon source for plant and living soil organisms. The amylase expression is influenced by different factors such as agricultural practices, type of plant, and environmental (Fioretto *et al.,* 2000). This study, amylase activity lacked sufficient resolution to discriminate the differences in soil managements because values of amylase activity in soil without organic amendment were in between soils with organic amendments (S2 and S3). Furthermore, soil with *Pueraria* cover crop (S3) had the highest amylase activity in rainy season while it had the lowest in dry season.

N-acetyl-glucosaminidase is one of chitinolytic enzyme. This enzyme involve in C and N cycling in soil because it degrade chitin to amino sugars which easily mineralize into C and N in soil (Ekenler and Tabatabai, 2002). In this study, N-acetyl-glucosaminidase activity cannot discriminate between soils with organic amendments (S2 and S3) and no organic amendment (S1) because values of this enzyme of soil without organic amendment (S1) were in between soils with organic amendments (S2 and S3). The highest N-acetyl-glucosaminidase activity in soil amended with *Pueraria* cover crop + organic input (S3) in rainy season may associate with highest soil organic carbon and total N content. It has been reported that higher N-acetyl-glucosaminidase activity was associated with higher soil organic carbon and total N content (Acosta-Martínez *et al.,* 2007). In contrast, in dry season, N-acetyl-glucosaminidase activity was the lowest in amended soil with *Pueraria* cover crop + organic input (S3). This season, the total N in soil with *Pueraria* cover crop + organic input (S3) was the lowest and this may be one of reason for the lowest values of N-acetyl-glucosaminidase in this soil.

Urease is an enzyme that catalyzes the hydrolysis of urea to NH_3 and CO_2. This enzyme is produced by large numbers of microorganisms in soil (Mobley and Hausinger, 1989). This investigation, urease activity was the least in soil with *Pueraria* cover crop + organic input (S3) in both rainy and dry seasons. High urease activity in soil with external organic input (S2) may due to addition of commercial compost, which may possibly contain high urease. It has been reported that urease enzyme was higher in the soil amended with composts than amended with fresh materials (Pascual *et al.,* 2002). Furthermore, this study found that urease activity was increased in dry season. This may occur form soil pH because urease activity is influenced by pH (Byrnes and Amberger, 1989). This study, the increase in soil pH in dry season may positively affect urease activity.

Principal component analysis on soil enzyme activities with the results obtained in rainy and dry seasons were constructed (Figure 11). In rainy season, PCA showed axis 1 and axis 2 for 65.1% and 15.4% of the variance, respectively. In dry season, PCA showed axis 1 and axis 2 for 45.7% and 28.3% of the variance, respectively. Testing the significance of groupings (sites) was carried out on 10,000 permutations and was significantly discriminated ($P<0.00$) in both seasons.

Principal component analysis showed that, in each season, the soil without amendment (S1) was characterized by low enzymatic activities. Enzyme activities were generally high in soil amended with *Pueraria* cover crop + external organic input (S3) in rainy season and were generally high in soil amended with external organic input (S2) in dry season. This indicated that enzyme tested could vary according to the season.

For example, soil with *Pueraria* cover crop + external organic input (S3) had significantly higher ($P<0.05$) in amylase and N-acetyl-glucosaminidase activities than soil with external organic input (S2) in rainy season while, in dry season, it had significantly lower ($P<0.05$). Bandick and Dick (1999) suggested that obstacle of using biological measurements as part of an index probably occurs from seasonal variations. They found that in the legume cover crop site, β-glucosidase activity changed significantly between seasons whereas FDA hydrolysis did not change. Collins et al. (1992) observed that microbial biomass C increased in Fall and decreased in Spring. Moscatelli et al. (2005) also suggested that time is the factor mostly affecting soil biological properties and nutrients availability.

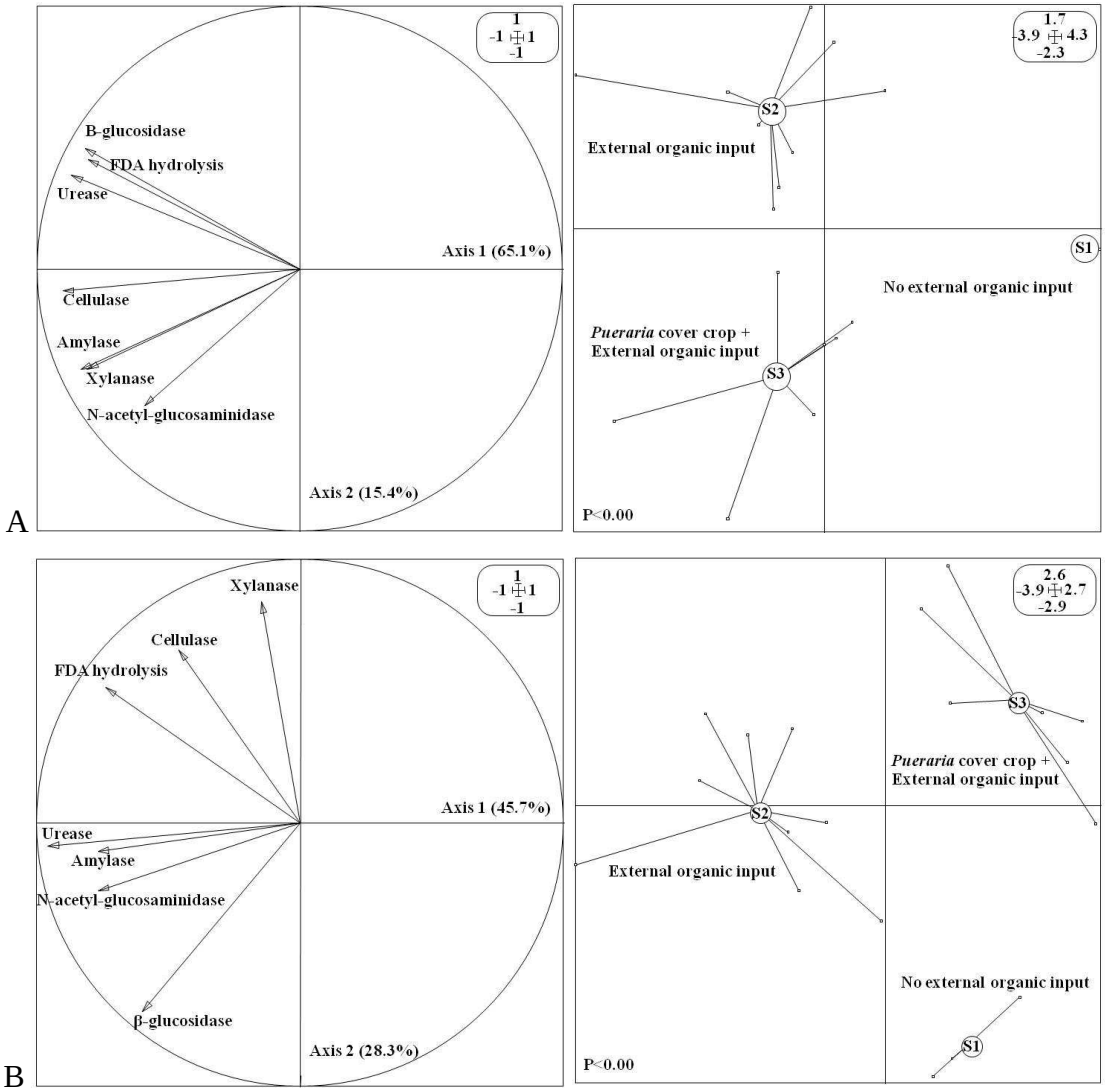


Figure 11 Principal component analysis showed relationship between soil enzyme activities and different organic managements during rainy (A) and dry (B) seasons.

The *Pueraria phaseoloides* is a leguminous and it can use as cover crop to enhance soil microbial biomass (Dinesh *et al.*, 2009). Studies have revealed that the soil with legume cover crops can increase soil enzyme activities such as β-glucosidase, cellulase and urease (Bandick and Dick, 1999; Dinesh *et al.*, 1999; Dinesh *et al.*, 2004). In this study, soil with *Pueraria* cover crop + organic input (S3) were lower in these enzymes activities than soil with external organic input (S2) in both in rainy and dry season. The period of soil amendment may be one factor as possible to explain this result. In this study, period of using *Pueraria* cover crop (approximately 3 years) may be inadequate to increase microbial

activities greater than other soils (S1 and S2). It has been reported that the soil with legume cover crop over 12 years was significantly increase in enzyme activities and microbial biomass (Dinesh *et al.*, 2004; Dinesh *et al.*, 2006). Bandick et al. (1999) found that enzyme activities were higher in soil with 6-year legume cover crop than soil without organic amendments.

The investigation of different enzyme activities together can provide information of status of soil processes (Acosta-Martínez *et al.*, 2003). The assay for cellulase and xylanase activities can be used as indicator to discriminate between soil with organic amendment and soil without organic amendment. Xylanase activity may be used to monitor effect of *Pueraria* cover crop because it was consistently high under soil with *Pueraria* cover crop (S3). In addition, FDA hydrolysis activity can applied to be indicator for impact of external organic materials used in this study.

However, the monitoring enzyme activity over time need for investigating activity change and eliminate problem in seasonal change. This can obtain potential of enzyme activities to be early indicators of soil management.

III.2.8 CONCLUSION

The biochemical and macrofaunal parameters measured can separate soils according to their management. Principal components analysis revealed that most enzymes tested were higher in soil with organic amendment (S2 or S3) than in soil without organic amendment (S1). The quality of external organic materials may effect on higher enzyme activities. In this study, xylanase and cellulase activities were high in soil with *Pueraria* cover crop + external organic input (S3). This indicates that these enzymes probably associate with quality of *Pueraria* residue and they may be used as indicators for consequence of *Pueraria* cover crop. In addition, especially dry season, most enzymes tested were lower in soil without organic amendment (S1) than in soils with organic amendments (S2 and S3). The organic materials used in this study probably maintain microbial processes.

The macrofauna in this study was scanty and patchy. This may due to soil property as sandy or quality of organic materials used as unpalatable for macrofauna. However, ants, termites, Arachnida and Diplopoda were higher in plantations with organic amendments (S2 and S3) than plantation without organic amendment (S1). The infertility of unamended soil may have effect on macrofauna abundance or diversity. These macroinvertebrates may sensitive to quality of soil and thus, they probably used for indicators of organic materials used in this study.

Since most enzyme activities and macrofauna densities were high in plantations with organic amendments (S2 or S3), organic matter and organic carbon contents in soil were high. However, soil with *Pueraria* cover crop + organic input (S3) had the highest organic matter and organic carbon. These properties of soil may lead to the highest *Hevea* productivity when compared with other soils (S1 and S2).

This study suggest that using *P. phaseoloides* as cover crop combined with organic materials can increase *Hevea* productivity and sustain long-term soil fertility because of higher diversity of organic materials. The *Pueraria* cover crop may stimulate *Hevea* growth at the early stage because of ability in N-fixation. Further studies are recommended to investigate organic materials chemistry and soil microbial communities as interacting controls on decomposition rate. These researches can obtain a better understanding of role of macrofauna and microorganisms in organic materials decomposition. Furthermore, to characterize the synchronization between nutrient availability of organic materials and *Hevea* nutrient demand may provide.

GENERAL CONCLUSION
AND
FUTURE PERSPECTIVES

General conclusion

This dissertation demonstrates that trunk phloem necrosis (TPN) seems to have effect on ecosystem of *Hevea* soil (Chapter III.1). The biological activities in soil were probably affected by TPN under rainy season, which is important period for organisms and plant growths. Enzyme activities were distinguished between soil with healthy tree and with TPN affected tree when it was analyzed by principle component analysis (PCA). High cellulase, xylanase and amylase activities were associated with healthy trees and were lower near diseased trees. The lower values of polysaccharides in soil under TPN affected tree may be due to increase in plant defensive chemistry, especially polyphenol, which accumulated in plant component of TPN affected tree. Under dry period, polysaccharides activities may be not inhibited by plant defensive mechanism because *Hevea* trees were not induced the stress by tapping, and therefore higher cellulase and xylanase were expressed in soil under TPN affected tree (B and BH). PCA also revealed that N-acetyl-glucosaminidase activity in rainy period was associated with soil under midway between TPN affected tree and healthy trees (BH). This may be also affected by plant defensive process. N-acetyl-glucosaminidase is one of chitinases and many plants can produce when they are in stressed situation.

Investigation of macrofauna revealed that termite group was the most abundant in soils under TPN affected trees (B and BH). Higher population of termite under zones of TPN probably associated with higher accumulated barks, which were shredded by disease attack.

Determining numeration of fungi showed that *Paecilomyces lilacinus* and *Trichoderma asperellum* were abundant in soil with TPN. The mechanism in tannins degradation may affect on the distribution of these fungi. The structure of soil microbial community obtained from similar dendrogram of 28S rDNA-DGGE showed that fungal cluster under TPN affected trees (B and BH) was clearly separated from fungal cluster under healthy trees (H and HH). The root chemical exudates, which produced from diseased trees and healthy tree, may influence microbial community.

In investigating the soil organic amendment (Chapter III.2), several trends were observed. Cellulase, β-glucosidase, urease and FDA hydrolysis activities in soil under *Hevea* plantation with organic input (S2) were higher than with *Pueraria* cover crop+ organic input (S3). Higher expression of these enzyme activities may be due to longer-period accumulation of exogenous organic materials, which added into plantation. The xylanase activity was consistently high in soil under plantation with *Pueraria* cover crop + organic input (S3). The macrofauna community was varied by season variation. Macrofauna abundance was the

highest under plantation with *Pueraria* cover crop + organic input (S3) in rainy period but it was the lowest in dry period. Coleoptera and Diplopoda abundances seem to associate with cover cropped plantation (S3). The investigation of soil properties revealed that organic carbon, organic matter and CEC were higher in soil under plantation with cover crop-organic input (S3) than with organic input alone (S2). The productivity of *Hevea* tree including growth rate and latex yield appears to increase under plantation with cover crop-organic input (S3). The capacity in N_2-fixation of *Pueraria* cover crop probably strongly influenced the trees growth at the early stage, which positively affected long-term latex production.

The organic carbon and organic matter content in soil were the lowest under plantation without organic management (S1) compared to plantation with organic management (S2 and S3) and were significantly lower than plantation with cover crop-organic input (S3). This fertility parameters of soil may indicates that the high apparition of TPN was associated with lower soil fertility. Therefore, this study can sustain the hypothesis that lower soil fertility is a partial determinant of TPN incident. Hence, this study is beneficial for *Hevea* grower and institute both of government and private. The obtained information from this study can be used as practical knowledge for development of *Hevea* production and protection of TPN apparition.

This study succeeded in the objectives and can conclude as follows:

1. The TPN disease of *H. brasiliensis* has effects on soil macrofauna, soil enzyme activities and soil microbial community in the rainy season. The low polysaccharidase (cellulase, xylanase and amylase) activities and high N-acetyl-glucosaminidase activity seem to be used as biological indicators of TPN.

2. Soil organic amendment using *Pueraria* cover crop + external organic input (which uesed in this study including eucalyptus barks and sugarcane filter cake) positively associate with high organic matter and organic carbon content in soil and has great influence on productivity of *H. brasiliensis* including tree growth, and latex yield

3. The lower soil fertility, which indicated by lower soil chemical properties and biological activities, associate with high incident of TPN disease.

Future perspectives

The further investigation which would be appropriate to pursue as follows:

1. Determination of role of *Hevea* secondary metabolites and root exudates.

The data presented in this study showed that reduction of polysaccharidase activities in rainy season were strongly associated with soils under TPN affected trees. The secondary metabolites produced by TPN affected tree may be a considerable factor to inhibit polysaccharidase activities. The tapping in wet season may induce tree to produce defensive chemistry. In addition, obtained data from fungal genetic analysis revealed that some fungi probably related root chemical exudation of TPN affected tree. Investigation of secondary metabolites and root exudates compared between TPN affected tree and healthy tree can verify the findings reported in this work.

2. Investigation of chemical component of organic material.

Agricultural residues are the important source of energy for soil organisms. Chemical compound of organic material have a great potential in regulation of biological activities in soil. Knowledge of organic residue characteristic added into plantation will give a better understanding about macrofauna and microbial communities in the ecosystem.

3. Investigation of dynamic soil enzymes.

Soil enzymes are sensitive to change in biochemical component of organic matter and organic carbon but their activities were controlled by several factors. Investigating soil enzyme activities before and after organic material addition, in addition, evaluating soil enzyme activities over time (week or month) will clarify the effect of soil organic management. Long-term study may warrant the determination of ecological consequences of changes in enzyme activities and microbial community

4. Determination of microbiological role in soil.

Microorganisms have greatly efficiency in organic degradation mechanism. Determining the role of microorganisms in the biogeochemical cycling of organic material would provide information to support the soil chemical property in this work. To determine the specific microbial populations affected by both soil organic management and TPN

disease, and how these microbes associate with the rate of C and N cycling, it will be helpful to study microbial community composition and function.

REFERENCES

Abawi G.S. and Widmer T.L. 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology, **15**: 37-47.

Abdalla M.M. and El-Khoshiban N.H. 2007. The Influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticium aestivum* cultivars. Journal of Applied Sciences Research, **3**: 2062-2074.

Abdul-Baki A.A. and Teasdale J.R. 1993. A no-tillage tomato production system using hairy vetch and subterranean clover mulches. HortScience, **28** 106-108.

Abdul-Halim R.K., Salih H.M., Ahmed A.A. and Abdul-Rahem A.M. 1988. Growth and development of maxipak wheat as affected by soil salinity and moisture levels. Plant and Soil, **112**: 255-259.

Abraham J. and Chudek J.A. 2008. Studies on litter characterization using ^{13}C NMR and assessment of microbial activity in natural forest and plantation crops' (teak and rubber) soil ecosystems of Kerala, India. Plant Soil, **303**: 265-273.

Abu-Hamdeh N.H. 2003. Compaction and subsoiling effects on corn growth and soil bulk density. Soil Science Society of America Journal, **67**: 1213-1219.

Acosta-Martínez V. and Tabatabai M.A. 2000. Enzyme activities in a limed agricultural soil. Biology and fertility of soils, **31**: 85-91.

Acosta-Martínez V. and Tabatabai M.A. 2001. Tillage and residue management effects on arylamidase activity in soils. Biology and fertility of soils, **34**: 21-24.

Acosta-Martínez V., Zobeck T.M., Gill T.E. and Kennedy A.C. 2003. Enzyme activities and microbial community structure of agricultural semiarid soils. Biology and fertility of soils, **38**: 216-227.

Acosta-Martínez V., Cruz L., Sotomayor-Ramírez D. and Pérez-Alegría L. 2007. Enzyme activities as a affected by soil properties and land use in a tropical watershed. Applied Soil Ecology, **35**: 35-45.

Agamuthu P. and Broughton W.J. 1985. Nutrient cycling within the developing oil palmlegume ecosystem. Agriculture Ecosystems and Environment, **13**: 111-123.

Agassi M., Shainberg I. and Morin J. 1981. Effect of Electrolyte Concentration and Soil Sodicity on Infiltration Rate and Crust Formation. Soil Science Society of America Journal, **45**: 848-851.

Akmal M., Wang H.-z., Wu J.-j., Xu J.-m.X. and Xu D.-f. 2005. Changes in enzymes activity, substrate utilization pattern and diversity of soil microbial communities under cadmium pollution Journal of Environmental Sciences, **17**: 802-807.

Akpan A.U., Edem S.O. and Ndaeyo N.U. 2007. Latex yield of rubber (*Hevea brasiliensis* Muell Argo) as influenced by clone planted and locations with varying fertility status. Journal of Agriculture and Social Sciences, **3**: 28-30.

Albrecht C., Laurent P. and Lapeyrie F. 1994. Eucalyptus root and shoot chitinases, induced following root colonization by pathogenic versus ectomycorrhizal fungi, compared on one- and two-dimensional activity gels. Plant Science, **100**: 157-164.

Alvarez R., Santanatogli O.J. and Garcîa R. 1995. Effect of temperature on soil microbial biomass and its metabolic quotient in situ under different tillage systems Biology and fertility of soils, **19**: 227-230.

Amezketa E. 1999. Soil aggregate stability: a review. Journal of Sustainable Agriculture, **14**: 83-151.

An S.S., Mentler A., Acosta-Martínez V. and Blum W.E.H. 2009. Soil microbial parameters and stability of soil aggregate fractions under different grassland communities on the Loess Plateau, China. Biologia, **64**: 424-427.

Anderson J.D. and Ingram J.S.I. 1993. Tropical soil biology and fertility: a handbook of methods. 2 ed. CAB International.

Anderson J.M., 1995: Soil organisms as engineers: microsite modulation of macroscale processes "Linking Species and Ecosystems". Ed. C. G. Jones, J. H. Lawton, pp 94-106.

Anderson J.M., Ineson P. and Huish S.A. 1983. Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. Soil Biology and Biochemistry, **15** 463-467.

Anderson J.M., Leonard M.A., Ineson P. and Huish S. 1985. Faunal biomass: a key component of a general model of nitrogen mineralization. Soil Biology and Biochemistry, **17** 735-737.

Andersson M., Kjøller A. and Struwe S. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology and Biochemistry, **36**: 1527-1537.

Anjum R., Ahmed A., Ullah R., Jahangir M. and Yousaf M. 2005. Effect of soil salinity/sodicity on the growth and yield of different varieties of cotton. International Journal of Agriculture and Biology, **7**: 606-608.

Arevalo L., Alegre J., Bandy D. and Szott L. 1998. The effect of cattle grazing on soil physical and chemical properties in a silvopastoral system in the Peruvian Amazon Agroforestry Systems, **40**: 109-124.

Armecin R.B., Seco M.H.P., Caintic P.S. and Milleza E.J.M. 2005. Effect of leguminous cover crops on the growth and yield of abaca (*Musa textilis* Nee) Industrial Crops and Products, **21**: 317-323.

Aulakh M.S., Wassmann R., Bueno C., Kreuzwieser J. and Rennenberg H. 2001. Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. Plant Biology, **3**: 139-148.

Ayres M.P., Clausen T.P., Jr. S.F.M., Redman A.M. and Reichardt P.B. 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology, **78**: 1696-1712.

Badiane N.N.Y., Chotte J.L., Pate E., Masse D. and Rouland C. 2001. Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Applied Soil Ecology, **18**: 229-238.

Bais H.P., Weir T.L., Perry L.G., Gilroy S. and Vivanco J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, **57**: 233-266.

Bajpai B. and Patil S. 1996. Tannin acyl hydrolase (EC 3.1.1.20) activity of *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma* World Journal of Microbiology and Biotechnology, **12**: 217-220.

Bajpai B. and Patil S. 1997. Induction of tannin acyl hydrolase (EC 3.1.1.20) activity in some members of fungi imperfecti. Enzyme and Microbial Technology, **20**: 612-614.

Bandick A.K. and Dick R.P. 1999. Field management effects on soil enzyme activities. Soil Biology and Biochemistry, **31**: 1471-1479.

Baptist F., Zinger L., Clement J.C., Gallet C., Guillemin R., Martins J.M., Sage L., Shahnavaz B., Choler P. and Geremia R. 2008. Tannin impacts on microbial diversity and the functioning of alpine soils: a multidisciplinary approach. Environmental Microbiology, **10**: 799-809.

Barahona R., Sanchez S., Lascano C.E., Owen E., Morris P. and Theodorou M.K. 2006. Effect of condensed tannins from tropical legumes on the activity of fibrolytic enzymes from the rumen fungus *Neocallimastyx hurleyensis*. Enzyme and Microbial Technology, **39**: 281-288.

Barbhuiya A.R., Arunachalam A., Pandey H.N., Arunachalam K., Khan M.L. and Nath P.C. 2004. Dynamics of soil microbial biomass C, N and P in disturbed and

undisturbed stands of a tropical wet-evergreen forest. European Journal of Soil Biology, **40**: 113-121.

Bardgett R.D. and Cook R. 1998. Functional aspects of soil animal diversity in agricultural grasslands. Applied Soil Ecology, **10**: 263-276.

Bardgett R.D. and Chan K.F. 1999. Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. Soil Biology and Biochemistry, **31**: 1007-1014.

Bardgett R.D. and Shine A. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology and Biochemistry, **31**: 317-321.

Bates S.T. and Garcia-Pichel F. 2009. A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: their diversity and relative contribution to microbial biomass. Environmental Microbiology, **11**: 56-67.

Battestin V. and Macedo G.A. 2007. Tannase production by *Paecilomyces variotii*. Bioresource technology, **98**: 1832-1837.

Berzsenyi Z., Gyorffy B. and Lap D. 2000. Effect of crop rotation and fertilization on maize and wheat yields and yield stability in a long-term experiment. European Journal of Agronomy, **13**: 225-244.

Beyer L.A., B. Sorge, CSchulten, H.-R. and Blume H.-P. 1996. Soil organic matter composition and pesticide bonding in sandy soils in relation to groundwater protection in the Northwest German Lower Plain. Biology and fertility of soils, **23**: 266-272.

Bhat M.Y., Muddin H. and Bhat N.A. 2009. Histological interactions of Paecilomyces lilacinus and Meloidogyne incognita on bitter gourd Journal of American Science, **5**: 8-12.

Bhat T.K., Makkar H.P.S. and Singh B. 1997. Preliminary studies on tannin degradation by *Aspergillus niger* van Tieghem MTCC 2425. Letters in Applied Microbiology, **25**: 22-23.

Binet F., Fayolle L. and Pussard M. 1998. Significance of earthworms in stimulating soil microbial activity. Biology and fertility of soils, **27**: 79-84.

Blair G.J. and Boland O.W. 1978. The release of phosphorus from plant material added to soil. Australian Journal of Soil Research, **16**: 101-111.

Blanchart E., Villenave C., Viallatoux A., Barthès B., Girardin C., Azontonde A. and Feller C. 2006. Long-term effect of a legume cover crop (*Mucuna pruriens* var. *utilis*)

on the communities of soil macrofauna and nematofauna, under maize cultivation, in southern Benin European Journal of Soil Biology, **42**: S136-S144.

Bokma E., H.T.J. R., M. S., B.W. D. and J.J. B. 2002. Expression and characterization of active site mutants of hevamine, a chitinase from the rubber tree Hevea brasiliensis. European Journal of Biochemistry, **269**: 893-901.

Bonanomi G., Chiurazzi M., Caporaso S., Del Sorbo G., Moschetti G. and Felice S. 2008. Soil solarization with biodegradable materials and its impact on soil microbial communities. Soil Biology and Biochemistry, **40**: 1989-1998.

Bonte D., Baert L. and Maelfait J.-P. 2002. Spider assemblage structure and stability in a heterogeneous coastal dune system (Belgium). The Journal of Arachnology, **30**: 331-343.

Borneman J., Skroch P.W., O'Sullivan K.M., Palus J.A., Rumjanek N.G., Jansen J.L., Nienhuis J. and Triplett E.W. 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. Applied and Environmental Microbiology, **62**: 1935-1943.

Bosatta E. and Agren G.I. 1997. Theoretical analyses of soil texture effects on organic matter dynamics. Soil Biology and Biochemistry, **29**: 1633-1638.

Bossio D.A., Girvan M.S., Verchot L., Bullimore J., Borelli T., Albrecht A., Scow K.M., Ball A.S., Pretty J.N. and Osborn A.M. 2005. Soil microbial community response to land use change in an agricultural landscape of western Kenya. Microbial ecology, **49**: 50-62.

Bossuyt H., Six J. and Hendrix P.F. 2002. Aggregate-protected carbon in no-tillage and conventional tillage agroecosytems using carbon-14 labled plant residue. Soil Science Society of America Journal, **66**: 1965-1973.

Bougoure D.S. and Cairney J.W.G. 2005. Fungi associated with hair roots of *Rhododendron lochiae* (Ericaceae) in an Australian tropical cloud forest revealed by culturing and culture-independent molecular methods. . Environ. Microbiol., **7**: 1743-1754.

Boyer J., Michellon R., Chabanne A., Reversat G. and Tibere R. 1999. Effects of trefoil cover crop and earthworm inoculation on maize crop and soil organisms in Reunion Island. Biology and fertility of soils, **28**: 364-370.

Bradley R.L., Titus B.D. and Preston C.P. 2000. Changes to mineral N cycling and microbial communities in black spruce humus after additions of $(NH_4)_2SO_4$ and condensed tannins extracted from *Kalmia angustifolia* and balsam fir. Soil Biology and Biochemistry, **32**: 1227-1240.

Bradshaw L. and Lanini W.T. 1995. Use of perennial cover crops to suppress weeds in Nicaraguan coffee orchards. International Journal of Pest Management, **4**: 185-194.

Broeckling C.D., Broz A.K., Bergelson J., Manter D.K. and Vivanco J.M. 2008. Root Exudates Regulate Soil Fungal Community Composition and Diversity. Applied and Environmental Microbiology, **74**: 738-744.

Bruggen A.H.C.V. and Semenov A.M. 2000. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology, **15**: 13-24.

Brunius G. 1980. Technical aspects of the use of 39,69-diacetylfluorescein for vital fluorescent staining of bacteria. Current Microbiology, **4**: 321-323.

Brussaard L., De Goede R.G.M., Hemerik L. and Veschoor B.C., 2005: Soil biodiversity: stress and change in grasslands under restoration succession "Biological diversity and function in soils". Ed. R. D. Bardgett, M. B. Usher, D. W. Hopkins, pp 333-362.

Bruyn L.A. 1999. Ants as bioindicators of soil function in rural environments. Agriculture, Ecosystems and Environment, **74**: 425-441.

Buchmann N. 2000. Biotic and abiotic factors controlling soil respiration rates in *Pieca abies* stands. Soil Biology and Biochemistry, **32**: 1625-1635.

Bugg R.L. and Waddington C. 1994. Using cover crops to manage arthropod pests of orchards: a review. Agriculture Ecosystems and Environment, **50**: 11-28.

Bultman T.L. and Letz G.W. 1982. Abundance and community structure of forest floor spiders following litter manipulation. Oecologia, **55**: 34-41.

Burns R.G. and Edwards J.A. 1979. Pesticide breakdown by soil enzymes. Pest Management Science, **11** 506-512.

Buschiazzo D.E., Aimar S.B. and Garcia Queijeiro J.M. 1999. Long-term maize, sorghum, and millet monoculture effects on an Argentina Typic Ustipsamment. Arid Land Research and Management, **13**: 1-15.

Buyer J.S. and Kaufman D.D. 1997. Microbial diversity in the rhizosphere of corn grown under conventional and low-input systems Applied Soil Ecology, **5**: 21-27.

Byrnes B.H. and Amberger A. 1989. Fate of broadcast urea in a flooded soil when treated with N-(n-butyl)thiophospheric triamide, a urease inhibitor. Fertility Research, **18**: 221-231.

Caesar-TonThat T.-C. and Cochran V.L. 2000. Soil aggregate stabilization by a saprophytic lignin-decomposing basidiomycete fungus I. Microbiological aspects. Biology and fertility of soils, **32**: 374-380.

Callisto M., Barbosa F.A.R. and Moreno P. 2002. The influence of *Eucalyptus* plantations on the macrofauna associated with *Salvinia auriculata* in southeast Brazil. Brazilian Journal of Biology, **62**: 63-68.

Carneiro R.M.D.G. and Cayrol J.-C. 1991. Relationship between inoculum density of the nematophagous fungus *Paecilomyces lilacinus* and control of *Meloidogyne arenaria* on tomato. Revue de Nématologie, **14**: 629-634.

Carof M., De Tourdonnet S., Coquet Y., Hallaire V. and Roger-Estrade J. 2007. Hydraulic conductivity and porosity under conventional and no-tillage and the effect of three species of cover crop in northern France. Soil Use and Management, **23**: 230-237.

Carter E.B., Theodorou M.K. and Morris P. 1999. Responses of *Lotus corniculatus* to environmental change. 2. Effect of elevated CO2, temperature and drought on tissue digestion in relation to condensed tannin and carbohydrate accumulation. Journal of the Science of Food and Agriculture, **79**: 1431-1440.

Carter M.R. 2002. Soil quality for sustainable land management: organic matter and aggregation interactions that maintain soil functions. Agronomy Journal, **94**: 38-47.

Carter M.R., Gregorich E.G., Angers D.A., Donald R.G. and Bolinder M.A. 1998. Organic C and N storage, and organic C fractions,in adjacent cultivated and forested soils of eastern Canada. Soil and Tillage Research, **47**: 253-261.

Cederbaum S.B., Carroll J.P. and Cooper R.J. 2004. Effects of alternative cotton agriculture on avian and arthropod populations. Conservation Biology, **18**: 1272-1282.

Cells R., Cornejo J., Hermosin M.C. and Koskinen W.C. 1997. Sorption-desorption of atrazine and simazine by model soil colloidal components. Soil Science Society of America Journal, **61**: 436-443.

Chander K., Goyal S., Mundra M.C. and Kapoor K.K. 1997a. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics Biology and fertility of soils, **24**: 306-310.

Chander K., Goyal S., Mundra M.C. and Kapoor K.K. 1997b. Organic matter, microbial biomass and enzyme activity of soils under different crop rotations in the tropics. Biology and fertility of soils, **24**: 306-310.

Chander K., Goyal S., Nandal D.P. and Kapoor K.K. 1998. Soil organic matter, microbial biomass and enzyme activities in a tropical agroforestry system. Biology and fertility of soils, **27**: 168-172.

Chaou H.I., Zibilske L.M. and Ohno T. 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability Soil Biology and Biochemistry, **35**: 295-302.

Chaudhuri P.S., Pal T.K., Bhattacharjee G. and Dey S.K. 2003. Rubber leaf litters (*Hevea brasiliensis* var. RRM 600) as vermiculture substrate for epigeic earthworm, *Perionyx excavatus*, *Eudrilus eugeniae* and *Eisenia fetida*. Pedobiologia, **47**: 796-800.

Chen W., Wu L., Frankenberger W.T.J. and Chang A.C. 2008. Soil enzyme activities of long-term reclaimed wastewater-irrigated soils. Journal of Environmental Quality, **37**: S36-S42.

Cherstin H., Sookmark U., Trouslot P., Pellegrin F. and Nandris D. 2004. Rubber tree (*Hevea* brasiliensis) bark necrosis syndrome III: a physiological disease linked to impaired cyanide metabolism. Plant Disease, **88**: 1047.

Chiarini L., Bevivino A., Dalmastri C., Nacamulli C. and Tabacchioni S. 1998. Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Applied Soil Ecology, **8**: 11-18.

Clarke H.R.G., Davis J.M., Wilbert S.M., Bradshaw Jr H.D. and Gordon M.P. 1994. Wound-induced and developmental activation of a poplar tree chitinase gene promoter in transgenic tobacco. Plant Molecular Biology, **25**: 799-815.

Clarke H.R.G., Lawrence S.D., Flaskerud J., Korhnak T.E., Gordon M.P. and DAVIS J.M. 1998. Chitinase accumulates systemically in wounded poplar trees. Physiologia Plantarum, **103**: 154-161.

Clermont-Dauphin C., Cabidoche Y.-M. and Meynard J.-M. 2004. Effects of intensive monocropping of bananas on properties of volcanic soils in the uplands of the French West Indies. Soil Use and Management, **20**: 105-113

Coley P.D. and Barone J.A. 1996. Herbivory and plant defenses in tropical forests. Annual Review of Ecology and Systematics, **27**: 305-335.

Collins H.P., Rasmussen P.E. and Douglas Jr C.L. 1992. Crop rotation and residue management effects on soil carbon and microbial dynamics. Soil Science Society of America Journal, **56**: 783-788.

Cosgrove L., McGeechan P.L., Robson G.D. and Handley P.S. 2007. Fungal communities associated with degradation of polyester polyurethane in soil. Applied and Environmental Microbiology, **73**: 5817-5824.

Crow W.T., Weingartner D.P., Dickson D.W. and Mcsorley R. 2001. Effect of sorghum-sudangrass and velvetbean cover crops on plant-parasitic nematodes associated with potato production in Florida. Supplement to the Journal of Nematology, **33**: 285-288.

da Silva R.H. and Rosolem C.A. 2003. Early development and nutrition of cover crop species as affected by soil compaction Journal of Plant Nutrition, **26**: 1635-1648.

Dai J., Huff D.R. and Schlossberg M.J. 2009. Salinity Effects on Seed Germination and Vegetative Growth of Greens-Type Poa annua Relative to Other Cool-Season Turfgrass Species. Crop Science, **49**: 696-703.

Dalal R.C. and Mayer R.J. 1987. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. VII. Dynamics of nitrogen mineralization potentials and microbial biomass. Australian Journal of Soil Research, **25** 461-472.

Dangerfield J.M. 1990. Abundance, biomass and diversity of soil macrofauna in savana woodland and associated managed habitats. Pedobiologia, **34**: 141-150.

Das M., Royer T.V. and Leff L.G. 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Applied and Environmental Microbiology, **73**: 756-767.

Daube J. and Wolters V. 2000. Microbial activity and functional diversity in the mounds of three different ant species Soil Biology and Biochemistry, **32**: 93-99.

de Caire G.Z., de Cano M.S., Palma R.M. and de Muléa C.Z. 2000. Changes in soil enzyme activities following additions of cyanobacterial biomass and exopolysaccharide. Soil Biology and Biochemistry, **32**: 1985-1987.

de Caire G.Z., de Cano M.S., de Mulé M.C.Z., Palma R.M. and Colombo K. 1997. Exopolysaccharide of *Nostoc muscorum* (Cyanobacteria) in the aggregation of soil particles. Journal of Applied Phycology, **9**: 249-253.

De Neve S. and Hofman G. 2000. Influence of soil compaction on carbon and nitrogen mineralization of soil organic matter and crop residues. Biology and fertility of soils, **30**: 544-549.

Decaëns T., Lavelle P., Jiineiiez Jaen J.J., Escobar G. and Rippstein G. 1994. Impact of land management on soil macrofauna in the Oriental Llanos of Colombia. European Journal of Soil Biology, **30**: 157-168.

Decker K.L.M., Boerner R.E.J. and Morris S.J. 1999. Scale-dependent patterns of soil enzyme activity in a forested landscape. Canadian Journal of Forest Research, **29**: 232-241.

Desjadins T., Charpentier F., Pashanasi B., Pando-Bahoun A., Lavelle P. and Mariotti. 2003. Effects of earthworm inoculation on soil organic matter dynamics of cultivated ultisol. Pedobiologia, **47**: 835-841.

Devi B.N. and Yadava P.S. 2006. Seasonal dynamics in soil microbial biomass C, N and P in a mixed-oak forest ecosystem of Manipur, North-east India Applied Soil Ecology, **31**: 220-227.

Di Battista C. 1997. Comportement en pépinière et en plantation d'un champignon ectomycorhizien *Laccaria bicolor* S238N inoculé sur Epicea (*Picea abies*) et Douglas (*Pseudotsuga menziesii*). Typage moléculaire de la souche introduite, 392p. Doctoral Thesis Université Henri-Poincaré, Nancy, France.

Dick R.P. 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. Agriculture, Ecosystems and Environment, **40**: 25-36.

Dick R.P., Myrold D.D. and Perle E.A. 1988. Microbial biomass and enzyme activities in compacted and rehabilitated skid trail. Soil Science Society of America Journal, **52**: 512-516.

Dilly O. and Blume H.P. 1998. Indicators to assess sustainable land use with reference to soil microbiology. Advances in GeoEcology, **31**: 29-39.

Dinesh R., Suryanarayana M.A., Ghoshal Chaudhuri S. and Sheeja T.E. 2004. Long-term influence of leguminous cover crops on the biochemical properties of a sandy clay loam Fluventic Sulfaquent in a humid tropical region of India. Soil and Tillage Research, **77**: 69-77.

Dinesh R., Chaudhuri G.S., Sheeja T.E. and Shiva K.N. 2009. Soil microbial activity and biomass is stimulated by leguminous cover crops. Journal of Plant Nutrition and Soil Science, **172**: 288-296.

Dinesh R., Suryanarayana M.A., Ghoshal Chaudhuri S., Sheeja T.E. and Shiva K.N. 2006. Long-term effects of leguminous cover crops on biochemical and biological properties in the organic and mineral layers of soils of a coconut plantation. European Journal of Soil Biology, **42**: 147-157.

Dinesh R., Suryanarayana M.A., Shyam Prasad G., Bandyopadhyay A.K., Nair A.K. and Sharma T.V.R.S. 1999. Influence of leguminous cover crops on microbial and selected enzyme activities in soils of a plantation. Journal of Plant Nutrition and Soil Science, **162**: 57-60.

Diouf M., Brauman A., Miambi E. and Rouland-Lefèvre C. 2005. Fungal communities of foraging soil sheeting built by several fungus-growing termite species (Isoptera,

Termitidae: Macrotermitinae) in dry savana (Thiès,Senegal). Sociobiology, **45**: 899-914.

Domingues C.M. and Peralta R.M. 1993. Production of amylase by soil fungi and partial biochemical characterization of amylase of a selected strain (*Aspergillus fumigatus* Fresenius). Canadian Journal of Microbiology, **39**: 681-685.

Dornbush M.E. 2007. Grasses, litter, and their interaction affect microbial biomass and soil enzyme activity. Soil Biology and Biochemistry, **39**: 2241-2249.

Driebe E.M. and Whitham T.G. 2000. Cottonwood hybridization affects tannin and nitrogen content of leaf litter and alters decomposition. Oecologia, **123**: 99-107.

Duineveld B.M., Kowalchuk G.A., Keijzer A. and van Elsas J.D. 2001. Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. Applied and Environmental Microbiology, **67**: 172-178.

Edenborn S.L. and Sexstone A.J. 2007. DGGE fingerprinting of culturable soil bacterial communities complements culture-independent analyses. Soil Biology and Biochemistry, **39**: 1570-1579.

Edwards C.A. and Lofty J.R. 1982. The effect of direct drilling and minimal cultivation on earthworm populations. Journal of Applied Ecology, **19**: 723-734.

Ekenler M. and Tabatabai M.A. 2002. Effects of trace elements on β-glucosaminidase activity in soils. Soil Biology and Biochemistry, **34**: 1829-1832.

Elad Y. and Kapat A. 1999. The role of *Trichoderma harzianum* protease in the bio-control of *Botrytis cinerea*. European Journal of Plant Pathology, **105**: 177-189.

Elliott L.F., Lynch J.M. and Papendick R.I., 1996: The microbial component of soil quality "Soil biochemistry". Ed. G. Stotzky, J. M. Bollag, pp 1-22.

Ellis R.J., Morgan P., Weightman A.J. and Fry J.C. 2003. Cultivation-dependent and -independent approaches for determining bacterial diversity in heavy-metal-contaminated soil. Applied and Environmental Microbiology, **69**: 3223-3230.

Emmerling C., Udelhoven T. and Schröder D. 2001. Response of soil microbial biomass and activity to agricultural de-intensification over a 10 year period. Soil Biology and Biochemistry, **33**: 2105-2114.

Epelde L., Becerril J.M., Hernandez-Allica J., Barrutia. O. and Garbisu C. 2008. Functional diversity as indicator of the recovery of soil health derived from *Thlaspi caerulescens* growth and metal phytoextraction. Applied Soil Ecology, **39**: 299-310.

Epron D., Nouvellon Y., Roupsard O., Mouvondy W., Mabiala A., Saint-André L., Joffre R., Jourdan C., Bonnefond J.M., Berbigier P. and Hamel O. 2004. Spatial and temporal variations of soil respiration in a Eucalyptus plantation in Congo. Forest Ecology and Management, **202**: 149-160.

Eslyn W.B., Kirk T.K. and Effland F.J. 1975. Changes in the chemical composition of wood caused by six soft-rot fungi. Phytopathology, **65**: 473-476.

Ewel J.J., Mazzarino M.J. and Berish C.W. 1991. Tropical Soil Fertility Changes Under Monocultures and Successional Communities of Different Structure. Ecological Applications, **1**: 289-302.

Eyles A., Davies N.W., Mitsunaga T., Mihara R. and Mohammed C. 2004. Role of *Eucalyptus globulus* wound wood extractives: evidence of superoxide dismutase-like activity. Forest pathology, **34**: 225-232.

Fall S., Brauman A. and Chotte J.-L. 2001. Comparative distribution of organic matter in particle and aggregate size fractions in the mounds of termites with different feeding habits in Senegal : *Cubitermes niokoloensis* and *Macrotermes bellicosus*. Applied Soil Ecology, **17**: 131-140.

Fang M., Motavalli P.P., Kremer R.J. and Nelson K.A. 2007. Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils. Applied Soil Ecology, **37**: 150-160.

FAO. 2009. FAOSTAT [Online] http://faostat.fao.org/default.aspx

Faterrigo J.M., Balser T.C. and Turner M.G. 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology, **87**: 570-579.

Feber R.E., Bell J., Johnson P.J., Firbank L.G. and Macdonald D.W. 1998. The effects of organic farming on surface-active spider (Araneae) assemblages in wheat in southern England, UK. The Journal of Arachnology, **26**: 190-202.

Fioretto A., Papa S., Curcio E., Sorrentino G. and Fuggi A. 2000. Enzyme dynamics on decomposing leaf litter of *Cistus incanus* and *Myrtus communis* in a Mediterranean ecosystem. Soil Biology and Biochemistry, **32**: 1847-1855.

Fliessbach A., Oberholzer H.-R., Gunst L. and Mader P. 2007. Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture Ecosystems and Environment, **118**: 273-284.

Fosu M., Kuhne R.F. and Vlek P.L.G. 2007. Mineralization and microbial biomass dynamics during decomposition of four leguminous residues. Journal of Biological Sciences, **7**: 632-637.

Franchini J.C., Pavan M.A. and Miyazawa M. 2004. Redistribution of phosphorus in soil through cover crop roots. Brazilian Archives of Biology and Technology, **47**: 381-386.

Frankenberger W.T.J. and Bingham F.T. 1982. Influence of salinity on soil enzyme activities. Soil Science Society of America Journal, **46**: 1173-1177.

Franzluebbers A.J. 2002. Water infiltration and soil structure related to organic matter and its stratification with depth. Soil and Tillage Research, **66**: 197-205.

Freixo A.A., Machado Plod, dos Santos H.P., Silva C.A. and Fadigas F.D. 2002. Soil organic carbon and fractions of a Rhodic Ferralsol under the influence of tillage and crop rotation systems in southern Brazil. Soil and Tillage Research, **64**: 221-230.

Frenkel H., Goertzen J.O. and Rhoades J.D. 1978. Effect of clay type and content, exchangeable sodium percentage, and electrolyte concentration on clay dispersion and soil hydraulic conductivity. Soil Science Society of America Journal, **142**: 32-39.

Freya S.D., Smitha H., Drijberb R. and Melilloc J. 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biology and Biochemistry, **40**: 2904-2907.

Frioni L., Dodera R., Malatés D. and Irigoyen I. 1998. An assessment of nitrogen fixation capability of leguminous trees in Uruguay. Applied Soil Ecology, **7**: 271-279.

Frouz J. 2008. The effect of litter type and macrofauna community on litter decomposition and organic matter accumulation in post-mining sites. Biologia, **63**: 249-253.

Frouz J., Elhottova D., Kuraz V. and Sourkova M. 2006a. Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites : Results of a field microcosm experiment. Applied Soil Ecology, **33**: 308-320.

Frouz J., Elhottová D., Kuráž V. and Šourková M. 2006b. Effects of soil macrofauna on other soil biota and soil formation in reclaimed and unreclaimed post mining sites: Results of a field microcosm experiment. Applied Soil Ecology, **33**: 308-320.

Fuentes J.P., Bezdicek D.F., Flury M., Albrecht S. and Smith J.L. 2006. Microbial activity affected by lime in a long-term no-till soil. Soil and Tillage Research, **88**: 123-131.

Gajda A. and Martyniuk S. 2005. Microbial biomass C and N and activity of enzymes in soil under winter wheat grown in different crop management systems. Polish Journal of Environmental Studies, **14**: 159-163.

Gajic B., Dugalic G. and Djurovic N. 2006. Comparison of soil organic matter content, aggregate composition and water stability of gleyic fluvisol from adjacent forest and cultivated areas. Agronomy Research, **4**: 499-508.

Gamble G.R., Akin D.E., Makkar H.P. and Becker K. 1996. Biological degradation of tannins in sericea lespedeza (*Lespedeza cuneata*) by the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus* analyzed by solid-state 13C nuclear magnetic resonance spectroscopy. Applied and Environmental Microbiology, **62**: 3600-3604.

Gamble G.R., Snook M.E., Henriksson G. and Akin D.E. 2000. Phenolic constituents in flax bast tissue and inhibition of cellulase and pectinase. Biotechnology Letters, **22**: 741-746.

Ganihar S.R. 2003. Nutrient mineralization and leaf litter preference by the earthworm *Pontoscolex corethrurus* on iron ore mine wastes. Restoration Ecology, **11**: 475-482.

Gao Z., Li B., Zheng C. and Wang G. 2008. Molecular Detection of Fungal Communities in the Hawaiian Marine Sponges *Suberites zeteki* and *Mycale armata*. Applied and Environmental Microbiology, **74**: 6091-6101.

García-Gil J.C., Plaza C., Soler-Rovira P. and Polo A. 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry, **32**: 1907-1913.

Garcia C. and Hermandez T. 1997. Biological and biochemical indications in derelict soils subject to erosion. Soil Biology and Biochemistry, **29**: 171-177.

Gaspar M.L., Cabello M.N., Pollero R. and Aon M.A. 2001. Fluorescein diacetate hydrolysis as a measure of fungal biomass in soil. Current Microbiology, **42** 339-344.

Gaspard J.T., Jaffee B.A. and Ferris H. 1990. Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. Journal of Nematology, **22**: 207-213.

Gasperi-Mago R.R. and Troeh F.R. 1979. Microbial effects on soil erodibility. Soil Science Society of America Journal, **43**: 765-768.

Gerber P.J., Heitmann J.A. and Joyce T.W. 1997. Purification and characterization of xylanases from *Trichoderma*. Bioresource technology, **61**: 127-140.

Gianfreda L., Rao M.A., Piotrowska A., Palumbo G. and Colombo C. 2005. Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution. Science of the Total Environment, **341**: 265-279.

Gibson W.P. and Burns R.G. 1977. The breakdown of malathion in soil and soil components Microbial ecology, **3**: 219-230.

Gidrol X., Chrestin H., Tan H.-L. and Kush A. 1994. Hevein, a lectin-like protein from *Hevea brasiliensis* (Rubber Tree) is involved in the coagulationo f latex. The Journal of Biological Chemistry, **269**: 9278-9283.

Giller K.E., Bignell D., Lavelle P., Swift M., Barrios E., Moreira F., Van Noordwijk M., Barios I., Karanja N. and Huising J., 2005: Soil biodiversity in rapidly changing tropical landscapes: scaling down and scaling up "Biological diversity and function in soils". Ed. R. D. Bardgett, M. B. Usher, D. W. Hopkins, pp 295-318.

Gilot C., Lavelle P., Blanchart E., Keli J., Kouassi P. and Guillaume G. 1995. Biological activity of soil under rubber plantations in Côte d'Ivoire. Acta Zoologica Fennica, **196**: 186-189.

Giordani R., Regli P. and Buc2 J. 2002. Antifungal effect of *Hevea brasiliensis* latex with various fungi. Its synergistic action with amphotericin B against *Candida albicans*. Mycoses, **45**: 476-481.

Gleeson D.B., Herrmann A.M., Livesley S.J. and Murphy D.V. 2008. Influence of water potential on nitrification and structure of nitrifying bacterial communities in semiarid soils Applied Soil Ecology, **40**: 189-194.

Golchin A., Clarke P., Oades J.M. and Skjemstad J.O. 1995. The effects of cultivation on the composition of organic matter and structural stability of soils. Australian Journal of Soil Research, **33**: 975-993.

Gonzalez G. and Zou X. 1999. Plant and litter influences on earthworm abundance and community structure in a tropical wet forest. Biotropica, **31** 486-493.

Gramss G., Voigt K.-D. and Kirsche B. 1999. Oxidoreductase enzymes liberated by plant roots and their effects on soil humic material Chemosphere, **38**: 1481-1494.

Green S.J., Stanley F., Hadar Y. and Minz D. 2004. Molecular tools for isolate and community studies of Pyrenomycete fungi. Mycologia, **96**: 439-451.

Green V.S., Stott D.E. and Diack M. 2006. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. Soil Biology and Biochemistry, **38**: 693-701.

Greene R.S.B., Kinnell P.I.A. and Wood J.T. 1994. Role of plant cover and stock trampling on runoff and soil-erosion from semi-arid wooded rangelands. Australian Journal of Soil Research, **32**: 953-973

Guedegbe H., Houngandan P., Roman J. and Rouland-Lefèvre C. 2008. Patterns of substrate degradation by some microfungi from fungus-growing termite combs (Isoptera: Termitidae: Macrotermiitnae). Sociobiology, **52**: 525-541.

Güsewell S. and Freeman C. 2005. Nutrient limitation and enzyme activities during litter decomposition of nine wetland species in relation to litter N : P ratios. Functional Ecology, **19**: 582-593.

Hagn A., Pritsch K., Schloter M. and Munch J.C. 2003. Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. Biology and fertility of soils, **38**: 236-244.

Hamer U., Makeschin F., Stadler J. and Klotz S. 2008. Soil organic matter and microbial community structure in set-aside and intensively managed arable soils in NE-Saxony, Germany. Applied Soil Ecology, **40**: 465-475.

Hance T., Gregoire-Wibo C. and Lebrun P. 1990. Agriculture and ground-beetle populations. Pedobiologia, **34**: 337-346.

Harris R.F., Chesters G., Allen O.N. and Attoe O.J. 1964. Mechanisms Involved in Soil Aggregate Stabilization by Fungi and Bacteria. Soil Science Society of America Journal, **28**: 529-532.

Hartz T.K., Johnstone P.R., Miyao E.M. and Davis R.M. 2005. Mustard cover crops are ineffective in suppressing soilborne disease or improving processing tomato yield. HortScience, **40**: 2016-2019.

Hättenschwiler S. and Vitousek P.M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends in Ecology and Evolution, **15**: 238-243.

Heneghan L., Coleman D.C., Zou X., Crossley D.A. and Haines B.L. 1999. Soil microarthropod contribution to decomposition dynamics: tropical-temperate comparisons of a single substrate. Ecology, **80**: 873-882.

Hernes P.J. and Hedges J.I. 2004. Tannin signatures of barks, needles, leaves, cones, and wood at the molecular level. Geochimica et Cosmochimica Acta, **68**: 1293-1307.

Hewlett T.E., Hewlett E.M. and Dickson D.W. 1997. Response of *Meloidogyne* spp., *Heterodera glycines*, and *Radopholus similis* to tannic acid. Supplement to the Journal of Nematology, **29**: 737-741.

Hewlett T.E., Dickson D.W., Mitchell D.J. and Mitchell M.E.K. 1988. Evaluation of *Paecilomyces lilacinus* as a biocontrol agent of *Meloidogyne javanica* on tobacco. Journal of nematology, **20**: 578-584.

Hietala A.M., Kvaalen H., Schmidt A., Jøhnk N., Solheim H. and Fossdal C.G. 2004. Temporal and spatial profiles of chitinase expression by Norway spruce in response to bark colonization by Heterobasidion annosum. Applied and Environmental Microbiology, **70**: 3948-3953.

Hinojosa M.B., Carreira J.A., García-Ruíz R. and Dick R.P. 2004. Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. Soil Biology and Biochemistry, **36**: 1559-1568.

Holland J.M., Perry J.N. and Winder L. 1999. The within field spatial and temporal distribution of arthropods within winter wheat. Bulletin of Entomological Research, **89**: 499-513.

Holland J.M., Cook S.K., Drysdale A., Hewitt M.V., Spink J. and Turley D. 1998. The impact on non-target arthropods of integrated compared to conventional farming: results from the LINK Integrated Farming Systems project. Brighton Crop Protection Conference - Pests and Disease, **2**: 625-630.

Holt J.A. 1998. Microbial activity in the mounds of some Australian termites. Applied Soil Ecology, **9**: 183-187.

Holt J.A. and Mayer R.J. 1998. Changes in microbial biomass and protease activities of soil associated with long-term sugar cane monoculture. Biology and fertility of soils, **27**: 127-131.

Hopkinson D. 1971. Leguminous cover crops for maintaining soil fertility in sisal in Tanzania II. effects on moisture status of soil and sisal. Experimental Agriculture, **7**: 337-343

Horn R., Way T. and Rostek J. 2003. Effect of repeated tractor wheeling on stress/strain properties and consequences on physical properties in structured arable soils Soil and Tillage Research, **73**: 101-106.

Houston A.P.C., Visser S. and Lautenschlager R.A. 1998. Microbial processes and fungal community structure in soils from clear-cut and unharvested areas of two mixedwood forests. Canadian Journal of Botany, **76**: 630-640.

Hu C. and Cao Z. 2007. Size and activity of the soil microbial biomass and soil enzyme activity in long-term field experiment. World Journal of Agricultural Sciences., **3**: 63-70.

Hu S., Coleman D., Beare M.H. and Hendrix P.F. 1995. Soil carbohydrates in aggrading and degrading agrosystems: influences of fungi and aggregates. Agriculture Ecosystems and Environment, **54** 77-88.

Hu S., Grunwald N.J., Van Bruggen A.H.C., Gamble G.R., Drinkwater L.E., Shennan C. and Demment M.W. 1997. Short-term effects of cover crop incorporation on soil carbon pools and nitrogen availability. Soil Science Society of America Journal, **61**: 901-911.

Huang B., Xu Y., Wu Y., Zhang S. and Chen X. 2008. Effect of root exudates of different resistant varieties of cucumber on fusarium wilt and preliminary studies on their resistance mechanism. Frontiers of Agriculture in China, **2**: 61-65.

Huhta V., Persson T. and Setälä H. 1998. Functional implications of soil fauna diversity in boreal forests. Applied Soil Ecology, **10**: 277-288

Hulugalle N.R., Lobry de Bruyn L.A. and Entwistle P. 1997. Residual effects of tillage and crop rotation on soil properties, soil invertebrate numbers and nutrient uptake in an irrigated Vertisol sown to cotton Applied Soil Ecology, **7**: 11-30.

Hyodo F., Tayasu I., Inoue T., Azuma J.-I., Kudo T. and Abe T. 2003. Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites (Macrotermitinae: Isoptera). Functional Ecology, **17**: 186-193.

Ibekwe A.M., Papiernik S.K., Gan J., Yates S.R., Yang C.-H. and Crowley D.E. 2001. Impact of Fumigants on Soil Microbial Communities Applied and Environmental Microbiology, **67**: 3245-3257.

Ikeda S., Ytow N., Ezura H., Minamisawa K. and Fujimura T. 2006. Soil microbial community analysis in the environmental risk assessment of transgenic plants. Plant Biotechnology, **23**: 137-151.

Ingels C.A., Scow K.M., Whisson D.A. and Drenovsky R.E. 2005. Effects of cover crops on grapevines, yield, juice composition, soil microbial ecology, and gopher activity. American journal of enology and viticulture, **56**: 19-29.

Ingham E.R. and Klein D.A. 1982. Relationship between fluorescein diacetate-stained hyphae and oxygen utilization, glucose utilization,and biomass of submerged fungal batch cultures. Applied and Environmental Microbiology, **44**: 363-370.

Ingram L.J., Schuman G.E., Stahl P.D. and Spackman L.K. 2005. Microbial respiration and organic carbon indicate nutrient cycling recovery in reclaimed soils. Soil Science Society of America Journal, **69**: 1737-1745.

Iovieno P., Morra L., Leone A., Pagano L. and Alfani A. 2009. Effect of organic and mineral fertilizers on soil respiration and enzyme activities of two Mediterranean horticultural soils Biology and fertility of soils, **45**: 555-561.

Isaac W.A.P., Brathwaite R.A.I., Ganpat W.G. and Bekele I. 2007. The impact of selected cover crops on soil fertility, weed and nematode suppression through farmer participatory research by fairtrade banana growers in St. Vincent and the Grenadines. World Journal of Agricultural Sciences, **3**: 371-379.

Jackson L.E. 2000. Fates and losses of nitrogen from a nitrogen-15-labeled cover crop in an intensively managed vegetable system. Soil Science Society of America Journal, **64**: 1404-1412.

Jensen S., Øvreås L., Daae F.L. and Torsvik V. 1998. Diversity in methane enrichments from agricultural soil revealed by DGGE separation of PCR amplified 16S rDNA fragments. FEMS Microbiology Ecology, **26**: 17-26.

Jeun Y.C. and Hwang B.K. 1991. Carbohydrate, amino acid, phenolic and mineral nutrient contents of pepper plants in relation to age-related resistance to *Phytophthora capsici*. Journal of Phytopathology, **131**: 40-52.

Jin K., Sleutel S., Buchan D., De Neve S., Cai D.X., Gabriels D. and Jin J.Y. 2009. Changes of soil enzyme activities under different tillage practices in the Chinese Loess Plateau Soil and Tillage Research, **104**: 115-120.

Joanisse G.D., Bradley R.L., Preston C.M. and Munson A.D. 2007. Soil enzyme inhibition by condensed litter tannins may drive ecosystem structure and processes: the case of *Kalmia angustifolia*. New Phytologist, **175**: 535-546.

Jocque R., Samu F. and Bird T. 2005. Density of spiders (Araneae: Ctenidae) in Ivory Coast rainforests. Journal of Zoology, **266**: 105-110.

Jordan D., Ponder F.J. and Hubbard V.C. 2003. Effects of soil compaction, forest leaf litter and nitrogen fertilizer on two oak species and microbial activity. Applied Soil Ecology, **23**: 33-41.

Jouquet P., Mamou L., Lepage M. and Velde B. 2002. Effect of termites on clay minerals in tropical soils: fungus-growing termites as weathering agents. European Journal of Soil Science, **53**: 521-527.

Jouquet P., Dauber J., Lagerlöf J., Lavelle P. and Lepage M. 2006. Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. Applied Soil Ecology, **32** 153-164.

Jouquet P., Bottinelli N., Lata J.-C., Mora P. and Caquineau S. 2007. Role of the fungus-growing termite *Pseudacanthotermes spiniger* (Isoptera, Macrotermitinae) in the dynamic of clay and soil organic matter content. An experimental analysis. Geoderma, **139**: 127-133.

Joyce B.A., Wallender W.W., Mitchell J.P., Huyck L.M., Temple S.R., Brostrom P.N. and Hsiao T.C. 2002. Infiltration and soil water storage under winter cover cropping in California's Sacramento Valley. Transactions of the ASAE, **45**: 315-326.

Juma N.J. 1993. Interrelationships between soil structure/texture, soilbiota/soil organic matter and crop production. Geoderma, **57**: 3-30.

Kaiser M., Ellerbrock R.H. and Gerke H.H. 2008. Cation exchange capacity and composition of soluble soil organic matter fractions. Soil Science Society of America Journal, **72**: 1278-1285.

Kandeler E., 1996: Ammonium "Methods in soil biology". Ed. Ö. R. Schinner F, Kandeler E, Margesin R, pp 406–408.

Kandeler E., Tscherko D. and Spiegel H. 1999. Long term monitoring of microbial biomass, N mineralization and enzyme activities of a Chernozem under different tillage management. Biology and fertility of soils, **28**: 343-351.

Kandeler E., Tscherko D., Bruce K.D., Stemmer M., Hobbs P.J., Bardgett R.D. and Amelung W. 2000. Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. Biology and fertility of soils, **32**: 390-400.

Kanokwiroon K., Teanpaisan R., Wititsuwannakul D., Hooper A.B. and Wititsuwannakul R. 2008. Antimicrobial activity of a protein purified from the latex of *Hevea brasiliensis* on oral microorganisms. Mycoses, **51**: 301-307.

Kasela S., Bennettb L.T. and Tibbits J. 2008. Land use influences soil fungal community composition across central Victoria, south-eastern Australia Soil Biology and Biochemistry, **40**: 1724-1732

Kaur B., Gupta S.R. and Singh G. 2000. Soil carbon, microbial activity and nitrogen availability in agroforestry systems on moderately alkaline soils in northern India. Applied Soil Ecology, **15**: 283-294.

Khaid M., Yang W.-j., Kishwar N., Rajput Z.I. and Garijo A. 2006. Study of cellulolytic soil fungi and two nova species and new medium. Journal of Zhejiang University SCIENCE B, **7**: 459-466.

Kirk J.L., Beaudette L.A., Hartb M., Moutoglis P., Klironomos J.N., Lee H. and Trevors J.T. 2004. Methods of studying soil microbial diversity. Journal of microbiological methods, **58**: 169-188.

Kladivko E.J. 2001. Tillage systems and soil ecology. Soil and Tillage Research, **61**: 61-76.

Klose S., Moore J.M. and Tabatabai M.A. 1999. Arylsulfatase activity of microbial biomass in soils as affected by cropping systems. Biology and fertility of soils, **29**: 46-54.

Kluczek-Turpeinen B., Tuomela M., Hatakka A. and Hofrichter M. 2003. Lignin degradation in a compost environment by the deuteromycete *Paecilomyces inflatus*. Applied Microbiology and Biotechnology, **61**: 374-379.

Kosola K.R., Dickmann D.I., Hall R.B. and Workmaster B.A.A. 2004. Cottonwood growth rate and fine root condensed tannin concentration. Tree Physiology, **24**: 1063-1068.

Kourtev P.S., Ehrenfeld J.G. and Huang W.Z. 2002. Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. Soil Biology and Biochemistry, **35**: 35-45.

Kraus T.E.C., Zasoski R.J. and Dahlgren R.A. 2004. Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. Plant Soil, **262**: 95-109.

Kumar T., Ghose M. and Brahmachary R.L. 2007. Effects of root exudates of two mangrove species on *in vitro* spore germination and growth of *Glomus mosseae*. Research Journal of Botany, **2**: 48-53.

Kuo S., Sainju U.M. and Jellum E.J. 1997. Winter cover crop effects on soil organic carbon and carbohydrate. Soil Science Society of America Journal, **61**: 145-152.

Lado M., Paz A. and Ben-Hur M. 2004. Organic matter and aggregate size interactions in infiltration, seal formation, and soil Loss. Soil Science Society of America Journal, **68**: 935-942.

Lal R. 1988a. Effects of macrofauna on soil properties in tropical ecosystems Agriculture, Ecosystems and Environment, **24**: 101-116.

Lal R. 1988b. Effects of macrofauna on soil properties in tropical ecosystems Agriculture Ecosystems and Environment, **24**: 101-116.

Lal R. and Kimble J.M. 1997. Conservation tillage for carbon sequestration. Nutrient Cycling in Agroecosystems, **49**: 243-253.

Laossi K.-R., Barot S., Carvalho D., Desjardins T., Lavelle P., Martins M., Mitja D., Rendeiro A.C., Rousseau G., Sarrazin M., Velasquez E. and Grimaldi M. 2008. Effects of plant diversity on plant biomass production and soil macrofauna in Amazonian pastures. Pedobiologia, **51**: 397-407.

Lavelle P. 1988. Earthworm activities and the soil system. Biology and fertility of soils, **6**: 237-251.

Lavelle P. 1997a. Faunal strategies and soil processes: adaptive strategies that determine ecosystem function. Advances in Ecological Research, **27**: 93-132.

Lavelle P. 1997b. Soil function in a changing world: the role of invertebrate ecosystem engineers. European Journal of Soil Biology, **33**: 159-193.

Lavelle P. and Spain A.V. 2001a. Invertebrate communities Kluwer Academic Publishers, Dordrecht, Boston & London.

Lavelle P. and Spain A.V. 2001b. Soil ecology Kluwer Academic Publishers, Dordrecht.

Lavelle P., Blanchart E., Martin A., Martin S., Spain A., Toutain F., Barois I. and Schaefer R. 1993. A hierarchical model for decomposition in terrestrial ecosystems: application to soils of the humid tropics. Biotropica, **25** 130-150.

Leckie S.E., Prescott C.E. and Grayston S.J. 2004. Forest floor microbial community response to tree species and fertilization of regenerating coniferous forests. Canadian Journal of Forest Research, **34**: 1426-1435.

Lee W.J., Wood C.W., Reeves D.W., Entry J.A. and Raper R.L. 1996. Interactive effects of wheel-traffic and tillage system on soil carbon and nitrogen. Communications in Soil Science and Plant Analysis, **27**: 3027-3043.

Leon M.C.C., Stone A. and Dick R.P. 2006. Organic soil amendments: impacts on snap bean common root rot (*Aphanomyes euteiches*) and soil quality. Applied Soil Ecology, **31**: 199-210.

Léonard J., Perrier E. and Rajot J.L. 2004. Biological macropores effect on runoff and infiltration: A combined experimental and modeling approach Agriculture, Ecosystems and Environment, **104**: 277-285.

Lerner A., Shor Y., Vinokurov A., Okon Y. and Jurkevitch E. 2006. Can denaturing gradient gel electrophoresis (DGGE) analysis of amplified 16s rDNA of soil bacterial populations be used in forensic investigations? Soil Biology and Biochemistry, **38**: 1188-1192.

Levy G.J., Goldstein D. and Mamedov A.I. 2005. Saturated hydraulic conductivity of semiarid soils: combined effects of salinity, sodicity, and rate of wetting. Soil Science Society of America Journal, **69**: 653-662.

Li X., Wu M. and Zhan Y. 2008. Effect of methamidophos on soil fungi community in microcosms by plate count, DGGE and clone library analysis Journal of Environmental Science, **20**: 619-625

Liang Z., Drijber R.A., Lee D.J., Dwiekat I.M., Harris S.D. and Wedin D.A. 2008. A DGGE-cloning method to characterize arbuscular mycorrhizal community structure in soil. Soil Biology and Biochemistry, **40**: 956-966.

Linares J., Scholberg J., Boote K., Chase C.A., Ferguson J.J. and Mcsorley R. 2008. Use of the cover crop weed index to evaluate weed suppression by cover crops in organic citrus orchards. HortScience, **43**: 27-34.

Lipiec J., Medvedev V.V., Birkas M., Dumitru E., Lyndina T.E., Rousseva S. and Fulajtár E. 2003. Effect of soil compaction on root growth and crop yield in Central and Eastern Europe. International Agrophysics, **17**: 61-69.

Liu M., Hu F., Chen X., Huang Q., Jiao J., Zhang B. and Li H. 2009. Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field: The influence of quantity, type and application time of organic amendments. Applied Soil Ecology, **42**: 166-175.

Liu X., Herbert S.J., Hashemi A.M., Zhang X. and Ding G. 2006. Effects of agricultural management on soil organic matter and carbon transformation – a review. Plant and Soil Environment, **52**: 531-543.

Lobry de Bruyn L.A. 1997. The status of soil macrofauna as indicators of soil health to monitor the sustainability of Australian agricultural soils. Ecological Economics, **23** 167-178.

Lobry de Bruyn L.A. and Conacher A.J. 1990. The role of termites and ants in soil modification - a review. Australian Journal of Soil Research, **28**: 55-93.

Lodge D.J. 1997. Factors related to diversity of decomposer fungi in tropical forests Biodiversity and Conservation, **6**: 681-688.

Lopez M.J., del Carmen Vargas-García M., Suárez-Estrella F. and Moreno J. 2006. Biodelignification and humification of horticultural plant residues by fungi. International Biodeterioration and Biodegradation, **57**: 24–30.

Loranger-Merciris G., Imbert D., Bernhard-Reversat F., Ponge J.-F. and Lavelle P. 2007. Soil fauna abundance and diversity in a secondary semi-evergreen forest in

Guadeloupe (Lesser Antilles): influence of soil type and dominant tree species Biology and fertility of soils, **44**.

Lorenz K., Preston C.M., Raspe S., Morrison I.K. and Feger K.H. 2000. Litter decomposition and humus characteristics in Canadian and German spruce ecosystems: information from tannin analysis and ^{13}C CPMAS NMR. Soil Biology and Biochemistry, **32**: 779-792.

Loveland P. and Webb J. 2003. Is there a critical level of organic matter in the agricultural soils of temperate regions: a review. Soil and Tillage Research, **70**: 1-18.

Lucas R.W., Casper B.B., Jackson J.K. and Balser T.C. 2007. Soil microbial communities and extracellular enzyme activity in the New Jersey Pinelands. Soil Biology and Biochemistry, **39** 2508-2519.

Lucasa R.W., Caspera B.B., Jacksonb J.K. and Balser T.C. 2007. Soil microbial communities and extracellular enzyme activity in the New Jersey Pinelands. Soil Biology and Biochemistry, **39**: 2508-2519.

Lundquist E.J., Jackson L.E., Scowa K.M. and Hsu C. 1999. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology and Biochemistry, **31**: 221-236.

Lupwayi N.Z., Clayton G.W., O'Donovan J.T., Harker K.N., Turkington T.K. and Soon Y.K. 2007. Phosphorus release during decomposition of crop residues under conventional and zero tillage. Soil and Tillage Research, **95**: 231-239.

Maarit-Niemi R., Heiskanen I., Wallenius K. and Lindstrom K. 2001. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. Journal of microbiological methods, **45**: 155-65.

Mahendran B., Raman N. and Kim D.J. 2006. Purification and characterization of tannase from *Paecilomyces variotii*: hydrolysis of tannic acid using immobilized tannase. Applied Microbiology and Biotechnology, **70**: 444-450.

Maheshwari R., Bharadwaj G. and Bhat M.K. 2000. Thermophilic fungi: their physiology and enzymes. Microbiology and Molecular Biology Reviews, **64**: 461-488.

Maier-Greiner U.H., Obermaier-Skrobranek B.M.M., Estermaier L.M., Kammerloher W., Freund C., Wulfing C., Burkert U., Matern D.H., Breuer M., Eulitz M., Kufrevioglu O.I. and Hartmann G.R. 1991. Isolation and properties of a nitrile hydratase from the soil fungus *Myrothecium verrucaria* that is highly specific for the

fertilizer cyanamide and cloning of its gene. Proceedings of the National Academy of Sciences, **88**: 4260-4264.

Makkar H.P.S., Singh B. and Kamra D.N. 1993. Biodegradation of tannins in oak (Quercus incana) leaves by Sporotrichum pulverulentum. Letters in Applied Microbiology, **18**: 39-41.

Mamedov A.I., Levy G.J., Shainberg I. and Letey J. 2001. Wetting rate, sodicity, and soil texture effects on infiltration rate and runoff. Australian Journal of Soil Research, **39**: 1293-1305.

Mandal D. and Tripathi K.P. 2009. Soil erosion limits for Lakshadweep Archipelago. Current Science, **96**: 276-280.

Mando A. 1997. Effect of termites and mulch on the soil physical rehabilitation of structurally crusted soils in the Sahel. Land Degradation and Development, **8**: 269-278.

Mando A., Stroosnijder L. and Brussaar L. 1996. Effects of termites on infiltration into crusted soil. Geoderma, **74**: 107-113.

Mapa R.B. and Gunsena H.P.M. 1995. Effect of alley cropping on soil aggregate stability of a tropical Alfisol. Agroforestry Systems, **32**: 237-245.

Marc P. and Canard A. 1997. Maintaining spider biodiversity in agroecosystems as a tool in pest control. Agriculture Ecosystems and Environment, **62**: 229-235.

Marosz A. 2004. Effect of Soil Salinity on Nutrient Uptake, Growth, and Decorative Value of Four Ground Cover Shrubs. Journal of Plant Nutrition, **27**: 977-989.

Marshall M.N., Cocolin L., Mills D.A. and VanderGheynst J.S. 2003. Evaluation of PCR primers for denaturing gradient gel electrophoresis analysis of fungal communities in compost. Journal of Applied Microbiology, **95**: 934-948.

Martens D.A. 2000. Management and crop residue influence soil aggregate stability. Journal of Environmental Quality, **29**: 723-727.

Martin M.N. 1991. The Latex of Hevea brasiliensis Contains High Levels of Both Chitinases and Chitinases/Lysozymes. Plant Physiol., **95**: 469-476.

Martínez-Iñigo M.J., Pérez-Sanza A., Ortiza I., Alonsoa J., Alarcóna R., Garcíaa P. and Loboa M.C. 2009. Bulk soil and rhizosphere bacterial community PCR–DGGE profiles and β-galactosidase activity as indicators of biological quality in soils contaminated by heavy metals and cultivated with *Silene vulgaris* (Moench) Garcke Chemosphere, **75**: 1376-1381.

Martinez-Raya A., Duran Zuazo V.H. and Francia-Martinez J.R. 2006. Soil Erosion and runoff response to plant-cover strips on semiarid slopes (SE Spain). Land Degradation and Development, **17** 1-11.

Martínez Á.T., Speranza M., Ruiz-Dueñas F.J., Ferreira P., Camarero S., Guillén F., Martínez M.J., Gutiérrez A. and del Río J.C. 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. International Microbiology, **8**: 195-204.

Martinez C.E. and Tabatabai M.A. 1997. Decomposition of biotechnology byproducts in soils. Journal of Environmental Quality, **26**: 625-632.

Matthews S., Mila I., Scalbert A. and Donnelly D.X. 1997. Extractable and non-extractable proanthocyanidins in barks. Phytochemistry, **45**: 405-410.

Mauch F., Mauch-Mani B. and Bolier T. 1988. Antifungal Hydrolases in Pea Tissue. II. Inhibition of Fungal Growth by Combinations of Chitinase and β-1,3-Glucanase. Plant Physiology, **88**: 936-942.

Mboukou-Kimbatsa I.M.C., Bernhard-Reversat F. and Loumeto J.J. 1998. Change in soil macrofauna and vegetation when fast-growing trees are planted on savanna soils Forest Ecology and Management, **110**: 1-12.

McIlvaine T.C. 1921. A buffer solution for colorimetric comparison. Journal of Biological Chemistry, **49**: 183-188.

McLatchey G.P. and Reddy K.R. 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. Journal of Environmental Quality, **27**: 1268-1274.

McVay K.A., Rascliffe D.E. and Hargrove W.L. 1989. Winter legume effects on soil properties and nitrogen fertilizer requirements. Soil Science Society of America Journal, **53**: 1856-1862.

Merlim A.d.O., Guerra J.G.M., Junqueira R.M. and de Aquino A.M. 2005. Soil macrofauna in cover crop of figs grown under organic management. Scientia Agricola, **62**: 57-61.

Meroth C.B., Hammes W.P. and Hertel C. 2003. Identification and Population Dynamics of Yeasts in Sourdough Fermentation Processes by PCR-Denaturing Gradient Gel Electrophoresis. Applied and Environmental Microbiology, **69**: 7453-7461.

Miller J.J., Beasley B.W., Larney F.J. and Olson B.M. 2005. Soil salinity and sodicity after application of fresh and composted manure with straw or wood-chips. Canadian Journal of Soil Science, **85**: 427-438.

Mobley H.L.T. and Hausinger R.P. 1989. Microbial urease: significance, regulation and molecular characterization. Microbiology Reviews, **53**: 85-108.

Möhlenhoff P., Müller L., Gorbushina A.A. and Petersen K. 2001. Molecular approach to the characterisation of fungal communities: methods for DNA extraction, PCR amplification and DGGE analysis of painted art objects. FEMS Microbiology Letters, **195**: 169-173.

Mora P., Miambi E., Jiménez J.J., Decaëns T. and Rouland C. 2005. Functional complement of biogenic structures produced by earthworms, termites and ants in the neotropical savannas. Soil Biology and Biochemistry, **37**: 1043-1048.

Morsy M.R., Jouve L., Hausman J.-F.-O., Hoffmann L. and Stewart J.M. 2007. Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. Journal of Plant Physiology, **164** 157-167.

Moscatelli M.C., Lagomarsino A., De Angelis P. and Grego S. 2005. Seasonality of soil biological properties in a poplar plantation growing under elevated atmospheric CO_2. Applied Soil Ecology, **30** 162-173.

Mougel C., Offre P.R., L., Corberand T., Gamalero E., Robin C. and Lemanceau P. 2006. Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. New Phytologist, **170**: 165-175.

Muhammad S., Müller T. and Joergensen R.G. 2008. Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab. Journal of Arid Environments, **72**: 448-457.

Mukuralinda A., Tenywa J.S., Verchot L., Obua J. and Namirembe S. 2009. Decomposition and phosphorus release of agroforestry shrub residues and the effect on maize yield in acidic soils of Rubona, southern Rwanda Nutrient Cycling in Agroecosystems, **84**: 155-166.

Muñoz-Carpena R., Ritter A., Bosch D.D., Schaffer B. and Potter T.L. 2008. Summer cover crop impacts on soil percolation and nitrogen leaching from a winter corn field Agricultural Water Management, **95**: 633-644.

Mutabaruka R., Hairiah K. and Cadisch G. 2007. Microbial degradation of hydrolysable and condensed tannin polyphenol-protein complexes in soils from different land-use histories. Soil Biology and Biochemistry, **39**: 1479-1492.

Nagayama K., Watanabe S., Kumakura K., Ichikawa T. and Makino T. 2007. Development and commercialization of *Trichoderma asperellum* SKT-1 (Ecohope®), a microbial pesticide. Journal of Pesticide Science, **32**: 141-142.

Nahmani J., Lavelle P. and Rossi J.P. 2006. Does changing the taxonomical resolution alter the value of soil macroinvertebrates as bioindicators of metal pollution? Soil Biology and Biochemistry, **38**: 385-396.

Nakatsu C.H. 2007. Soil microbial community analysis using denaturing gradient gel electrophoresis. Soil Science Society of America Journal, **71**: 562-571.

Nakatsu C.H., Torsvik V. and Øvreas L. 2000. Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. Soil Science Society of America Journal, **64**: 1382-1388.

Nandris D., Moreau R., Pellegrin F. and Chrestin H. 2004. Rubber tree (*Hevea brasiliensis*) bark necrosis syndrome II: First comprehensive report on causal agent stresses. Plant Disease, **88**: 1047.

Nandris D., Moreau R., Pellegrin F. and Chrestin H. 2005. Rubber tree bark necrosis: advances in symptomatology, etiology, epidemiology and causal factors of a physiological trunk disease. Tropical Agriculture Science and Technology, **28**: 1-8.

Nandris D., Moreau R., De Fay E., Masson A., Pellegrin F. and Chrestin H., 2006: Etiological investigations on trunk phloem necrosis of the rubber tree: biotic or abiotic causal factors? "Tapping panel dryness of rubber trees". Ed. J. Jacob, R. Krishnakumar, N. M. Mathew, pp 85-92.

Nandris D., Chrestin H., Noirot M., Nicole M., Thouvenel J.C., Rio B. and Geiger J.P. 1991a. La nécrose du phloème du tronc de l'hévéa en Côte d'Ivoire : 1. Symptomatologie et caractéristiques biochimiques. European Journal of Forest Pathology, **21**: 325-339.

Nandris D., Thouvenel J.C., Chrestin H., Nicole M., Gianotti J., Declert C., Rio B. and Noirot M. 1991b. La nécrose du phloème du tronc de l'hévéa en Côte d'Ivoire : 2. Etiologie de la maladie. European Journal of Forest Pathology, **21**: 340-353.

Naseby D.C., Moënne-Loccoz Y., Powell J., O'Gara F. and Lynch J.M. 1998. Soil enzyme activities in the rhizosphere of field-grown sugar beet inoculated with the biocontrol agent *Pseudomonas fluorescens* F113. Biology and fertility of soils, **27**: 39-43.

Ndiaye D., lensi R., Lepage M. and Brauman A. 2004. The effect of the soil-feeding termite Cubitermes niokoloensis on soil microbial activity in a semi-arid savanna in West Africa. Plant and Soil, **259**: 277-286.

Ndiaye E.L., Sandeno J.M., McGrath D. and Dick R.P. 2000. Integrative biological indicators for detecting change in soil quality. American Journal of Alternative Agriculture, **15**: 26-36.

Neave P. and Fox C.A. 1998. Response of soil invertebrates to reduced tillage systems established on a clay loam soil Applied Soil Ecology, **9**: 423-428.

Nelson D.R. and Mele P.M. 2006. The impact of crop residue amendments and lime on microbial community structure and nitrogen-fixing bacteria in the wheat rhizosphere. Australian Journal of Soil Research, **44**: 319-329.

Nelson N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry, **153**: 375-380.

Neves C.S.V.J., Feller C., Guimaraes M.F., Medina C.C., Tavares J. and Fortier M. 2003. Soil bulk density and porosity of homogeneous morphological units identified by the Cropping Profile Method in clayey Oxisols in Brazil. Soil and Tillage Research, **71**: 109-119.

Nichols-orians C. 1991. Condensed tannins, attine ants, and the performance of a symbiotic fungus Journal of Chemical Ecology, **17**: 1177-1195.

Nicole M., Thouvene J.C., Giannotti J., H. C., Geiger J.P., Nandris D. and Rio B. 1991. The histology of *Hevea brasiliensis* phloem necrosis. European Journal of Forest Pathology, **21**: 27-35.

Niemi R.M., Heiskanen I., Ahtiainen J.H., Rahkonen A., Mäntykoski K., Welling L., Laitinen P. and Ruuttunen P. 2009. Microbial toxicity and impacts on soil enzyme activities of pesticides used in potato cultivation Applied Soil Ecology, **41**: 293-304.

Nierop K.G.J., Preston C.M. and Verstraten J.M. 2006. Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization. A case study using four tannins. Soil Biology and Biochemistry, **38**: 2794-2802.

Nilsson K.S., Hyvonen R. and Agren G.I. 2005. Using the continuous-quality theory to predict microbial biomass and soil organic carbon following organic amendments. European Journal of Soil Science, **56**: 397-405.

Njunie M.N., Wagger M.G. and Luna-Orea P. 2004. Residue decomposition and nutrient release dynamics from two tropical forage legumes in a Kenyan environment. Agronomy Journal, **96**: 1073-1081.

Nomura M. and Itioka T. 2002. Effects of synthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Applied Entomology and Zoology, **37**: 285-289.

Norris T.B., Wraith J.M., Castenholz R.W. and McDermott T.R. 2002. Soil microbial community structure across a thermal gradient following a geothermal heating event. Applied and Environmental Microbiology, **68**: 6300-6309.

Oliveira A. and Pampulha M.E. 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. Journal of Bioscience and Bioengineering, **102**: 157-161.

Onemli F. 2004. The effects of soil organic matter on seedling emergence in sunflower (*Helianthus annuus* L.). Plant and Soil Environment, **50**: 494-499.

Oorts K., Vanlauwe B. and Merckx R. 2003. Cation exchange capacities of soil organic matter fractions in a Ferric Lixisol with different organic matter inputs Agriculture Ecosystems and Environment, **100**: 161-171.

Øvreås L., Jensen S., Daae F.L. and Torsvik V. 1998. Microbial community changes in a perturbed agricultural soil investigated by molecular and physiological approaches. Applied and Environmental Microbiology, **64**: 2739-2742.

Pabin J., Lipie J., Włodeka S., Biskupskia A. and Kausc A. 1998. Critical soil bulk density and strength for pea seedling root growth as related to other soil factors. Soil and Tillage Research, **46**: 203-208.

Painuli D.K. and Abrol I.P. 1986. Effects of exchangeable sodium on crusting behavior of a sandy loam soil. Australian Journal of Soil Research, **24**: 367-376.

Parijs J.V., Broekaert W.F., Goldstein I.J. and Peumans W.J. 1991. Hevein: an antifungal protein from rubber-tree (Hevea brasiliensis) latex. Planta, **183**: 258-264.

Parkin T.B. and Berry E.C. 1999. Microbial nitrogen transformations in earthworm burrows Soil Biology and Biochemistry, **31**: 1765-1771.

Pascual J.A., Gracia C. and Hemandez T. 1999. Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. Biology and fertility of soils, **30**: 1-6.

Pascual J.A., Moreno J.L., Hernández T. and C. G. 2002 Persistence of immobilised and total urease and phosphatase activities in a soil amended with organic wastes. Bioresource technology, **82**: 73-78.

Patrick W.H., Haddon C.B. and Hendrix J.A. 1957. The effects of longtime use of winter cover crops on certain physical properties of commerce loam. Soil Science Society of America, **21**: 366-368.

Pavarina E.C., Sette L.D., Anazawa T.A. and Durrant1 L.R. 1999. Enzymes produced by soil fungi following microaerobic growth on lignocellulosic materials Applied Biochemistry and Biotechnology, **82**: 153-163.

Pellegrin F., D. N. and Chrestin H. 2004. Rubber tree (*Hevea brasiliensis*) bark necrosis syndrome: 1. still no evidence of a biotic agent. Plant Disease, **88**: 1046.

Pellegrin F., Duran-Vila N., Musnster M.V. and Nandris D. 2007a. Ruber tree (*Hevea brasiliensis*) truk phloem necrosis: aetiological investigations failed to confirm any biotic causal agent. Forest pathology, **37**: 9-21.

Persiani A.M., Maggi O., Casado M.A. and Pineda F.D. 1998. Diversity and variability in soil fungi from a disturbed tropical rain forest. Mycologia, **90**.

Peterson C.A., Enstone D.E. and Taylor J.H. 1999. Pine root structure and its potential significance for root function. Plant Soil, **217**: 205-213.

Peyrard N., Pellegrin F., Chadoeuf J. and Nandris D. 2006. Statistical analysis of the spatio-temporal dynamic of rubber tree (*Hevea brasiliensis*) trunk phloem necrosis: no evidence of pathogen transmission. Forest pathology, **36**: 360-371.

Pinamonti F. 1998. Compost mulch effects on soil fertility, nutritional status and performance of grapevine. Nutrient Cycling in Agroecosystems, **51**: 239-248.

Pinto G.A.S., Leite S.G.F., Terzi S.C. and Couri S. 2001. Selection of tannase-producing *Aspergillus niger* strains. Brazilian Journal of Microbiology, **32**: 24-26.

Piotrowska-Seget Z., Cycoń M. and Kozdrój J. 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil Applied Soil Ecology, **28**: 237-246.

Pitkänen J. and Nuutinen V. 1998. Earthworm contribution to infiltration and surface runoff after 15 years of different soil management Applied Soil Ecology, **9**: 411-415.

Plante A.F., Conant R.T., Stewart C.E., Paustian K. and Six J. 2006. Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. Soil Science Society of America Journal, **70**: 287-296.

Porteous L.A., Seidler R.J. and Watrud L.S. 1997. An improved method for purifying DNA from soil for polymerase chain reaction amplification and molecular ecology applications. Molecular Ecology, **6**: 787-791.

Potthoff M., Steenwerth K.L., Jackson L.E., Drenovsky R.E., Scow K.M. and Joergensen R.G. 2006. Soil microbial community composition as affected by

restoration practices in California grassland. Soil Biology and Biochemistry, **38** 1851-1860.

Purseglove J.W. 1987. Tropical Crops: Dicotyledons Longman Group Ltd., London.

Radford B.J., Wilson-Rummenie A.C., Simpson G.B., Bell K.L. and Ferguson M.A. 2001. Compacted soil affects soil macrofauna populations in a semi-arid environment in central Queensland Soil Biology and Biochemistry, **33**: 1869-1872.

Rae C.M. and Castro T.F. 1967. Root exudates of the rice plant in relation to akagare, a physiological disorder of rice Plant and Soil, **26**: 317-323.

Rakwal R., G. Y. and Komatsu S. 2004. Chitinase induced by jasmonic acid, methyl jasmonate, ethylene and protein phosphatase inhibitors in rice. Molecular Biology Reports, **31**: 113-119.

Ramoliya P.J., Patel H.M. and Pandey A.N. 2006. Effect of salinization of soil on growth and nutrient accumulation in seedlings of *Prosopis cineraria*. Journal of Plant Nutrition, **29**: 283-303.

Ramoliya P.J., Patel H.M., Thakarar N.K. and Pandey A.N. 2004. Soil salinity and water status affect growth of seedlings of *Butea monosperma*. Tropical Ecology, **45**: 333-336.

Rasmussen P.H., Knudsen I.M.B., Elmholt S. and Jensen D.F. 2002. Relationship between soil cellulolytic activity and suppression of seedling blight of barley in arable soil. Applied Soil Ecology, **19**: 91-96.

Rea J.H., Wratten S.D., Sedcole R., Cameron P.J., Davis S.I. and Chapman R.B. 2002. Trap cropping to manage green vegetable bug *Nezara viridula* (L.) (Heteroptera: Pentatomidae) in sweet corn in New Zealand Agricultural and Forest Entomology, **4**: 101-107.

Reddy M.V. and Pasha M. 1993. Influence of rainfall, temperature and somes oil physico-chemical variables on seasonal population structure and vertical distribution of earthworms in two semi-arid tropical grassland soils International Journal of Biometeorology, **37**: 19-26.

Reed J.P., Krueger H.R. and Hall F.R. 1989. Fluorescein diacetate hydrolysis for determination of accelerated degradation of thiocarbamate herbicides Bulletin of Environmental Contamination and Toxicology, **43**: 929-934.

Reeves D.W. 1997. The role of soil organic matter in maintaining soil quality in continuous cropping systems. Soil and Tillage Research, **43**: 131-167.

Rhine E.D., Fuhrmann J.J. and Radosevich M. 2003. Microbial community responses to atrazine exposure and nutrient availability: Linking degradation capacity to community structure. Microbial ecology, **46**: 145-160.

Rietz D.N. and Haynes R.J. 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity Soil Biology and Biochemistry, **35**: 845-854.

Robertson E.B., Shlomo S. and Firestone M.K. 1991. Cover crop management of polysaccharide-mediated aggregation in a orchard soil. Soil Science Society of America Journal, **55**: 734-739.

Rodíguez-Kábana R. 1982. The effects of crop rotation and fertilization on soil xylanase activity in a soil of the southeastern United States Plant and Soil, **64**: 237-247.

Roldán A., Salinas-García J.R., Alguacil M.M., Díaz E. and Caravaca F. 2005. Soil enzyme activities suggest advantages of conservation tillage practices in sorghum cultivation under subtropical conditions Geoderma, **129**: 178-185.

Rosolem C.A., Foloni J.S.S. and Tiritan C.S. 2002. Root growth and nutrient accumulation in cover crops as affected by soil compaction. Soil and Tillage Research, **65**: 109-115.

Rouland-Lefevre C., Diouf M.N., Brauman A. and Neyra M. 2002. Phylogenetic relationships in Termitomyces (Family Agaricaceae) based on the nucleotide sequence of ITS: a first approach to elucidate the volutionary history of the symbiosis between fungus-growing termites and their fungi. Molecular Phylogenetics and Evolution, **22**: 423-429.

Rouland C., Lenoir F. and Lepage M. 1991. The role of symbiotic fungus in the digestive metabolism of several species of fungus-growing termites. Comparative Biochemistry and Physiology A, **99** 657-663.

Rouland C., Civas A., Renoux J. and Petek F. 1988. Synergistic activities of the enzymes involved in cellulose degradation, purified from *Macrotermes mülleri* and from its symbiotic, fungus *Termitomyces* sp. Comparative Biochemistry and Physiology B, **91**: 459-465.

Ruan H., Li Y. and Zou X. 2005. Soil communities and plant litter decomposition as influenced by forest debris: Variation across tropical riparian and upland sites. Pedobiologia, **49**: 529-538.

Rudall P. 1987. Laticifers in Euphorbiaceae: a conspectus. Botanical Journal of the Linnean Society, **94**: 143-163.

Rutigliano F.A., Virzo De Santo A., Berg B., Alfani A. and Fioretto A. 1996. Lignin decomposition in decaying leaves of *Fagus sylvatica* L. and needles of *Abies alba* Mill. . Soil Biology and Biochemistry, **28**: 101-106.

Ryckeboer J., Mergaert J., Vaes K., Klammer S., de Clercq D., Coosemans J., Insam H. and Swings J. 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Annals of Microbiology, **53**: 349-410.

Saffigna P.G., Powlson D.S., Brookes P.C. and Thomas G.A. 1989. Influence of sorghum residues and tillage on soil organic matter and soil microbial biomass in an Australian vertissol. Soil Biology and Biochemistry, **21**: 759-765.

Sainju U.M., Singh B.P. and Yaffa S. 2002. Soil organic matter and tomato yield following tillage, cover cropping, and nitrogen fertilization. Journal of Agronomy, **94**: 594-602.

Sainju U.M., Singh B.P., Rahman S. and Reddy V.R. 2000. Tillage, cover cropping, and nitrogen fertilization influence tomato yield and nitrogen uptake. HortScience, **35**: 217-221.

Saliha B.B., Krishnakumar S., Saravanan A. and Natarajan S.K. 2005. Microbial and Enzyme Dynamics in Distillery Spentwash Treated Soil. Research Journal of Agriculture and Biological Sciences, **1**: 166-169.

Sannino F. and Gianfreda L. 2001. Pesticide influence on soil enzymatic activities. Chemosphere, **45**: 417-425.

Santos I.S., Cunhab M.D., Machadoc O.L.T. and Gomesa V.M. 2004. A chitinase from *Adenanthera pavonina* L. seeds: purification, characterisation and immunolocalisation. Plant Science, **167**: 1203-1210.

Sardans J. and Peñuelas J. 2005. Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. Soil Biology and Biochemistry, **37**: 455-461.

Sardans J., Peñuelas J. and Estiarte M. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology, **39**: 223-235.

Sardinha M., Müller T., Schmeisky H. and Joergensen R.G. 2003. Microbial performance in soils along a salinity gradient under acidic conditions Applied Soil Ecology, **23**: 237-244.

Sawada K., Funakawa S. and Kosaki T. 2009. Different effects of pH on microbial biomass carbon and metabolic quotients by fumigation-extraction and substrate-induced respiration methods in soils under different climatic conditions Soil Science and Plant Nutrition, **55**: 363-374.

Scalbert A. 1991. Antimicrobial properties of tannins. Phytochemistry, **30**: 3875-3883.

Schädler M. and Brandl R. 2005. Do invertebrate decomposers affect the disappearance rate of litter mixtures? . Soil Biology and Biochemistry, **37**: 329-337.

Scheu S. 1987. Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biology and fertility of soils, **5**: 230-234.

Scheu S. 1990. Changes in microbial nutrient status during secondary succession and its modification by earthworms. Oecologia, **84**: 351-358.

Schinner F. 1982. Soil microbial activities and litter decomposition related to altitude Plant and Soil, **65**: 87-94.

Schlecht-Pietsch S., Wagner U. and Anderson T.-H. 1994. Changes in composition of soil polysaccharides and aggregate stability after carbon amendments to different textured soils. Applied Soil Ecology, **1**: 145-154.

Schmidt O., Curry J.P., Purvis G. and Clements R.O. 2001. Earthworm communities in conventional wheat monocropping and low-input wheat-clover intercropping systems. Annals of Applied Biology, **138**: 377-388.

Schnürer J. and Rosswall T. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied and Environmental Microbiology, **43**: 1256-1261.

Schofield J.A., Hagerman A.E. and Harold A. 1998. Loss of tannins and other phenolics from willow leaf litter. Journal of Chemical Ecology, **24**: 1409-1421.

Schultz P. and Urban N.R. 2008. Effects of bacterial dynamics on organic matter decomposition and nutrient release from sediments: a modeling study. Ecological Modelling, **210**: 1-14.

Scullion J. and Malik A. 2000. Earthworm activity affecting organic matter, aggregation and microbial activity in soils restored after opencast mining for coal. Soil Biology and Biochemistry, **32**: 119-126.

Seastedt T.R. 1984. The role of microarthropods in decomposition and mineralization processes. Annual Review of Entomology, **29**: 25-46.

Sekiguchi H., Hasegawa H., Okada H., kushida A. and Takenaka S. 2008. Comparative analysis of environmental variabiliy and fungal community structure in soil between organic and conventional commercial farms of cherry tomato in Japan. Microbes Environ., **23**: 57-65.

Senesi N., D'Orazio V. and Miano T.M. 1995. Adsorption mechamisms of *s*-triazine and bipyridylium herbicides on humic acids from hop field soils. Geoderma, **66**: 273-283.

Seo J.H. and Lee H.J. 2008. Mineral nitrogen effects of hairy vetch (*Vicia villosa* Roth) on maize (*Zea mays* L.) by green manure amounts. Journal of Agronomy, **7**: 272-276.

Shakir H.S. and Dindal L.D. 1997. Density and biomass of earthworms in forest and herbaceous microecosystems in central New York, North America. Soil Biology and Biochemistry, **29**: 275-285.

Sharon E., Chet I., Viterbo A., Bar-Eyal M., Nagan H., Samuels G.J. and Spiegel Y. 2007. Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix European Journal of Plant Pathology, **118**: 247-258.

Shelton A.M. and Badenes-Perez E. 2006. Concepts and applications of trap cropping in pest management. Annual Review of Entomology, **51**: 285-308.

Shukla M.K., Lal R. and Ebinger M. 2003. Tillage effects on physical and hydrological properties of a typic aggriquoll in central Ohio. Soil Science, **168**: 802-811.

Siddique T., Okeke B.C., Zhang Y., Arshad M., Han S.K. and Frankenberger W.T.J. 2005. Bacterial diversity in selenium reduction of agricultural drainage water amended with rice straw. Journal of Environmental Quality, **34**: 217-226.

Sigler W.V. and Turcob R.F. 2002. The impact of chlorothalonil application on soil bacterial and fungal populations as assessed by denaturing gradient gel electrophoresis. Applied Soil Ecology, **21**: 107-118.

Sileshi G. and Mafongoya P.L. 2006a. Long-term effects of improved legume fallows on soil invertebrate macrofauna and maize yield in eastern Zambia. Agriculture, Ecosystems and Environment, **115**: 69-78.

Sileshi G. and Mafongoya P.L. 2006b. Variation in macrofaunal communities under contrasting land use systems in eastern Zambia. Applied Soil Ecology, **33**: 49-60.

Silver W.L., Neff J., McGroddy M., Veldkamp E., Keller M. and Cosme R. 2000. Effects of soil texture on belowground carbon and nutrient storage in a lowland amazonian forest ecosystem. Ecosystems, **3**: 193-209.

Sinha A., Gupta S.R. and Rana R.S. 1986. Effect of soil salinity and soil water availability on growth and chemical composition of *Sorghum halepense* L. . Plant and Soil, **95**: 411-418.

Sinsabaugh R.L. and Linkins A.E. 1987. Inhibition of the *Thrichoderma viride* cellulase complex by leaf litter extracts. Soil Biology and Biochemistry, **19**: 719-725.

Skinner R.J. and Todd A.T. 1998. Twenty-five years of monitoring pH and nutrient status of soils in England and Wales. Soil Use and Management, **14**: 162-169.

Smith K.P. and Goodman R.M. 1999. Host variation for interactions with beneficial plant-associated microbes. Annu. Rev. Phytopathol., **37**: 473-491.

Somogyi M. 1945. Determination of blood sugar. The Journal of Biological Chemistry, **160**: 61-68.

Sparling G.P., Shepherd T.G. and Schipper L.A. 2000. Topsoil characteristics of three contrasting New Zealand soils under four long-term land uses. . New Zealand Journal of Agricultural Research, **43**: 569-583.

Spencer C.M., Cai Y., Martin R., Gaffney S.H., Goulding P.N., Magnolato D., Lilley T.H. and Haslam E. 1988. Polyphenol complexation-some thoughts and observations. Phytochemistry, **27**: 2397-2409.

Sroka K. and Finch O.-D. 2006. Ground beetle diversity in ancient woodland remnants in north-western Germany (Coleoptera, Carabidae) Journal of Insect Conservation, **10**: 335-350.

Stadler B., Schramm A. and Kalbitz K. 2006. Ant-mediated effects on spruce litter decomposition, solution chemistry, and microbial activity Soil Biology and Biochemistry, **38**: 561-572.

Stark C.H., Condron L.M., O'Callaghan M., Stewart A. and Di H.J. 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. Soil Biology and Biochemistry, **40**: 1352-1363.

Stark C.H.E., Condron L.M., Stewart A., Di H.J. and O'Callaghan M. 2007. Influence of organic and mineral amendments on soil microbial properties and processes. Applied Soil Ecology, **35**: 79-93.

Steenwerth K.L., Jackson L.E., Calderón F.J., Stromberg M.R. and Scow K.M. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology and Biochemistry, **34**: 1599-1611.

Ström L., Olsson T. and Tyler G. 1994. Differences between calcifuge and acidifuge plants in root exudation of low-molecular organic acids. Plant and Soil, **167**: 239-245.

Suganya S. and Sivasamy R. 2006. Moisture retention and cation exchange capacity of sandy soil as influenced by soil additives. Journal of Applied Sciences Resrach, **2**: 949-951.

Sun H.Y., Deng S.P. and Raun W.R. 2004. Bacterial community structure and diversity in a century-old manure-treated agroecosystem. Applied and Environmental Microbiology, **70**: 5868-5874.

Sung J.-K., Lee S.-M., Lee Y.-H., Choi D.-H., Kim T.-W. and Song B.-H. 2008. Impacts of cover crops on early growth, nitrogen uptake and carbohydrate composition of pepper plants. Korean Journal of Soil Science and Fertilizer, **41**: 44-49.

Tang X.-L., Zhou G.-Y., Liu S.-G., Zhang D.-Q., Liu S.-Z., Li J. and Zhou C.-Y. 2006. Dependence of soil respiration on soil temperature and soil moisture in successional forests in Southern China. Journal of Integrative Plant Biology, **48**: 654-663.

Teklay T. 2004. Seasonal dynamics in the concentrations of micronutrients and organic constituents in green and senesced leaves of three agroforestry species in southern Ethiopia. Plant and Soil, **267**: 297-307.

Thioulouse J., Chessel D., Dolédec S. and Olivier J.M. 1996. ADE-4 : a multivariate analysis and graphical display software. Statistics and Computing, **7**: 75-83.

Thomas C.F.G., Parkinson L. and Marshall E.J.P. 1998. Isolating the components of activity-density for the carabid beetle *Pterostichus melanarius* in farmland. Oecologia, **106**: 103-112.

Thompson I.P., Bailey M.J., Ellis R.J., Maguire N. and Meharg A.A. 1998. Response of soil microbial communities to single and multiple doses of an organic pollutant Soil Biology and Biochemistry, **31**: 95-105.

Tian G., Brussaard L. and Kang B.T. 1995. Breakdown of plant residues with contrasting chemical compositions under humid tropical conditions: effects of earthworms and millipedes. Soil Biology and Biochemistry, **27** 277–280.

Tilak K.V.B.R., Ranganayaki N., Pal K.K., De R., Saxena A.K., Nautiyal C.S., Mittal S., Tripathi A.K. and Johri B.N. 2005. Diversity of plant growth and soil health supporting bacteria. Curr. Sci., **89**: 136-150.

Timper P., Davis R.F. and Tillman P.G. 2006. Reproduction of *Meloidogyne incognita* on winter cover crops used in cotton production. Journal of nematology, **38**: 83-89.

TonThat T.C.C. and Cochran V.L. 2000. Soil aggregate stabilization by a saprophytic lignin-decomposing basidiomycete fungus. I. Microbiological aspects. Biology and fertility of soils, **32**: 374-380.

Torbert H.A. and Wood C.W. 1992. Effects of soil compaction and water-filled pore space on soil microbial activity and N losses. Communications in Soil Science and Plant Analysis, **23**: 1321-1331.

Torsvik V., Goksoyr J. and Daae F.L. 1990. High diversity in DNA of soil bacteria. Applied and Environmental Microbiology, **56**: 782-787.

Torsvik V., Daae F.L., Sandaa R.-A. and Ovreas L. 1998. Review article: novel techniques for analysing microbial diversity in natural and perturbed environments. Journal of Biotechnology, **64**: 53-62.

Trabue S.L., Palmquist D.E., Lydick T. and Koch Singles S. 2006. Effects of soil storage on the microbial community and degradation of metsulfuron-methyl. Journal of agricultural and food chemistry, **54**: 142-151.

Trásar-Cepeda C., Leiros M.C. and Gil-Sotres F. 2000. Biochemical properties of acid soils under climax vegetation (Atlantic oakwood) in an area of the European temperate-humid zone (Galicia, NW Spain): specific parameters. Soil Biology and Biochemistry, **32**: 747-755.

Tribak M., Ocampo J.A. and Garcia-Romera I. 2002. Production of xyloglucanolytic enzymes by *Trichoderma viride*, *Paecilomyces farinosus*, *Wardomyces inflatus*, and *Pleurotus ostreatus*. Mycologia, **94**: 404-410.

Tripathi S., Kumari S., Chakraborty A., Gupta A., Chakrabarti K. and Bandyapadhyay B.K. 2006. Microbial biomass and its activities in salt-affected coastal soils. Biology and fertility of soils, **42**: 273-277.

Tripathy R. and Singh A.K. 2004. Effect of water and nitrogen management on aggregate size and carbon enrichment of soil in rice-wheat cropping systems. Journal of Plant Nutrition and Soil Science, **167**: 216-228.

Vagen T.-G., Shepherd K.D. and Walsh M.G. 2006. Sensing landscape level change in soil fertility following deforestation and conversion in the highlands of Madagascar using Vis-NIR spectroscopy. Geoderma, **133**: 281-294.

van der Wal A., van Veen J.A., Pijl A.S., Summerbell R.C. and de Boer W. 2006. Constraints on development of fungal biomass and decomposition processes during restoration of arable sandy soils. Soil Biology and Biochemistry, **38**: 2890-2902.

van Elsas J.D., Frois-Duarte G., Keijzer-Wolters A. and Smit E. 2000. Analysis of the dynamics of fungal communities in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis. Journal of microbiological methods, **43**: 133-151.

VandenBygaart A.J., Fox C.A., Fallow D.J. and Protz R. 2000. Estimating Earthworm-Influenced Soil Structure by Morphometric Image Analysis. Soil Science Society of America Journal, **64**: 982-988.

Velasquez E., Lavelle P. and Andrade M. 2007. GISQ, a multifunctional indicator of soil quality. Soil Biology and Biochemistry, **39**: 3066-3080.

Venkateswarlu B., Srinivasarao C., Ramesh G., Venkateswarlu S. and Katyal J.C. 2007. Effects of long-term legume cover crop incorporation on soil organic carbon, microbial biomass, nutrient build-up and grain yields of sorghum/sunflower under rain-fed conditions. Soil Use and Management, **23**: 100-107.

Vepsäläinen M., Kukkonen S., Vestberg M., Sirviö H. and Niemi R.M. 2001. Application of soil enzyme activity test kit in a field experiment. Soil Biology and Biochemistry, **33**: 1665-1672.

Vepsäläinen M., Erkomaa K., Kukkonen S., Vestberg M., Wallenius K. and Niemi R. 2004. The impact of crop plant cultivation and peat amendment on soil microbial activity and structure Plant and Soil, **264**: 273-286.

Verma T.S. and Neue H.U. 1984. Effect of soil salinity level and zinc application on growth, yield, and nutrient composition of rice Plant and Soil, **82**: 3-14.

Vesterager J.M., Osterby S., Jensen E.S. and Schjoerring J.K. 1995. Symbiotic N_2-fixation by the cover crop *Pueraria phaseoloides* as influenced by litter mineralization. Plant and Soil, **177**: 1-10.

Vidal S., Salmon J.-M., Williams P. and Pellerin P. 1999. *Penicillium daleae*, a soil fungus able to degrade rhamnogalacturonan II, a complex pectic polysaccharide Enzyme and Microbial Technology, **24**: 283-290.

Virzo De Santo A., Berg B., Rutigliano F.A., Alfani A. and Fioretto A. 1993. Factors regulating early-stage decomposition of needle litter in five different coniferous forests. Soil Biology and Biochemistry, **25**: 1423-1433.

Vohland K. and Schroth G. 1999. Distribution patterns of the litter macrofauna in agroforestry and monoculture plantations in central Amazonia as affected by plant species and management. Applied Soil Ecology, **13** 57-68.

Waldrop M.P., Zak D.R., Blackwood C.B., Curtis C.D. and Tilman D. 2006. Resource availability controls fungal diversity across a plant diversity gradient. Ecology Letters, **9**: 1127-1135.

Walker T.S., Bais H.P., Grotewold E. and Vivanco J.M. 2003. Root exudation and rhizosphere biology. Plant Physiology, **132**: 44-51.

Wander M.M., Traina S.J., Stinner B.R. and Peters S.E. 1994. Organic and conventional management effects on biologically active soil organic matter pools. Soil Science Society of America Journal, **58**: 1130-1139.

Warkentin B.P. 1995. The changing concept of soil quality. Journal of Soil Water Conservation, **50**: 226-22.

Warren M.W. and Zou X. 2002. Soil macrofauna and litter nutrients in three tropical tree plantations on a disturbed site in Puerto Rico. Forest Ecology and Management, **170**: 161-171.

Wattel-Koek E.J.W., van Genuchten P.P.L., Buurman P. and van Lagen B. 2001. Amount and composition of clay-associated soil organic matter in a range of kaolinitic and smectitic soils Geoderma, **99**: 27-49.

White T.J., Bruns T., S. L. and Taylor L., 1990: Amplification and direct sequencing of fungal RNA genes for phylogenetics "PCR protocols, a guide for methods and applications". Ed. M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, pp 315-322.

Wichern J., Wichern F. and Joergensen R.G. 2006. Microbial performance in soils along a salinity gradient under acidic conditions. Geoderma, **137**: 100-108.

Williams J.D. 2004. Effects of long term winter wheat, summer fallow residue and nutrient management on field hydrology for a silt loam in north-central Ohio. Soil and Tillage Research, **75**: 109-119.

Willison T.W., O'Flaherty M.S., Tlustos P., Goulding K.W.T. and Powlson D.S. 1997. Variations in microbial populations in soils with different methane uptake rates. Nutrient Cycling in Agroecosystems, **49**: 85-90.

Wong V.N.L., Dalal R.C. and Greene R.S.B. 2008. Salinity and sodicity effects on respiration and microbial biomass of soil. Biology and fertility of soils, **44**: 943-953.

Wood T.G. 1988. Termites and the soil environment. Biology and fertility of soils, **6**: 228-236.

Wright A.L., Hons F.M. and Matocha J.E. 2005. Tillage impacts on microbial biomass and soil carbon and nitrogen dynamics of corn and cotton rotations. Applied Soil Ecology, **29**: 85-92.

Wu F. and Wang X. 2007. Effect of monocropping and rotation on soil microbial community diversity and cucumber yield and quality under protected cultivation. ISHS Acta Horticulturae, **761**: 555-561.

Wurzburger N. and Hendrick R.L. 2007. Rhododendron thickets alter N cycling and soil extracellular enzyme activities in southern Appalachian hardwood forests. Pedobiologia, **50**: 563-576.

Wyland L.J., Jackson L.E., Chaney W.E., Klonsky K., Koike S.T. and Kimple B. 1996. Altering surface soil dynamics with cover crops in a vegetable cropping system:

Impacts on yield, nitrate leaching, pests and management costs. Agriculture Ecosystems and Environment, **59**: 1-17.

Yagi R., Ferreira M.E., Cruz M.C.P.d. and Barbosa J.C. 2003. Organic matter fractions and soil fertility under the influence of liming, vermicompost and cattle manure. Scientia Agricola, **60**: 549-557.

Yang C.-H. and Crowley D.E. 2000. Rhizosphere Microbial Community Structure in Relation to Root Location and Plant Iron Nutritional Status Applied and Environmental Microbiology, **66**: 345-351.

Yang L.X., Pan J.J. and Yuan S.F. 2006. Predicting dynamics of soil organic carbon mineralization with a double exponential model in different forest belts of China. Journal of Forestry Research, **17** 39-43.

Yankelevich S.N., Fragoso C., Newton A.C. and Heal O.W. 2007. Successional changes in soil, litter and macroinvertebrate parameters following selective logging in a Mexican Cloud Forest. Applied Soil Ecology, **35**: 340-355.

Yao H., Jiao X. and Wu F. 2006. Effects of continuous cucumber cropping and alternative rotations under protected cultivation on soil microbial community diversity. Plant and Soil, **284**: 195-203.

Youn H.S. and Jung C. 2008. Effect of trap cropping on the egg parasitism of *Riptortus clavatus* (Thunberg) in soybean fields Journal of Asia-Pacific Entomology, **11**: 73-76

Yu Z. and Dahlgren R.A. 2000. Evolution of methods for measuring polyphenols in conifer foliage. Journal of Chemical Ecology, **26**: 2119-2140.

Zablotowicz R.M., Locke M.A. and Gaston L.A. 2007. Tillage and cover effects on soil microbial properties and fluometuron degradation. Biology and fertility of soils, **44**: 27-35.

Zhang B., Zhang H., Jin B., Tang L., Yang J., Li B., Zhuang G. and Bai Z. 2008. Effect of cypermethrin insecticide on the microbial community in cucumber phyllosphere Journal of Environmental Sciences, **20**: 1356-1362.

Zhang B.G.R., C., Lattaud C. and Lavelle P. 1993. Activity and origin of digestive enzymes in gut of the tropical earthworm *Pontoscolex corethrums*. European Journal of Soil Biology, **29** 7-11.

Zhang J.H., Mao Z.Q., Wang L.Q. and Shu H.R. 2007. Bioassay and identification of root exudates of three fruit tree species. Journal of Integrative Plant Biology, **49**: 257-261.

Zhang Q. and Zak J.C. 1998. Effects of water and nitrogen amendment on soil microbial biomass and fine root production in a semi-arid environment in West Texas. Soil Biology and Biochemistry, **30**: 39-45.

Zheng S., Yao J., Zhao B. and Yu Z. 2007. Influence of agricultural practices on soil microbial activity measured by microcalorimetry European Journal of Soil Biology, **43**: 151-157.

Zou X. 1993. Species effects on earthworm density in tropical tree plantations in Hawaii. Biology and fertility of soils, **15**: 35-38.

Zuazo V.H.D. and Pleguezuelo C.R.R. 2008. Soil-erosion and runoff prevention by plant covers. A review. Agronomy for Sustainable Development, **28**: 65-86.

Zuazo V.H.D., Martínez J.R.F., Pleguezuelo C.R.R., Raya A.M. and Rodríguez B.C. 2006. Soil-erosion and runoff prevention by plant covers in a mountainous area (se spain): Implications for sustainable agriculture The Environmentalist, **26**: 309-319.

Zuccaro A., Schoch C.L., Spatafora J.W., Kohlmeyer J., Draeger S. and Mitchell J.I. 2008. Detection and identification of fungi intimately associated with the brown seaweed *Fucus serratus*. Applied and Environmental Microbiology, **74**: 931-941.

Zuccarob A., Schulza B. and Mitchell J.I. 2003. Molecular detection of ascomycetes associated with *Fucus serratus*. Mycological Research, **107**: 1451-1466.

APPENDICES

Appendix 1 Density of the macrofauna (mean and standard deviation, individual m^{-2}) in four different sampling zones; near collar of affected tree (B), midway between affected trees and healthy trees (BH), near collar of healthy trees (H) and midway between healthy trees (HH) during rainy period of 2006 and dry period of 2008

.	Litter								Soil							
	Rainy				Dry				Rainy				Dry			
	B	BH	H	HH	B	BH	H	HH	B	BH	H	HH	B	BH	H	HH
Coleoptera (total)	**0.30**	**0.70**	**0.35**	**0.85**	**0.45**	**0.55**	**0.35**	**0.35**	**0.00**	**1.20**	**0.40**	**2.00**	**0.00**	**0.40**	**0.00**	**0.00**
	0.42	0.63	0.41	0.53	0.72	0.93	0.34	0.34	0.00	1.93	1.26	2.83	0.00	1.26	0.00	0.00
Carabidae sp.	**0.05**	**0.05**	**0.00**	**0.20**	**0.10**	**0.20**	**0.00**	**0.05**	**0.00**	**0.00**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.16	0.16	0.00	0.35	0.21	0.35	0.00	0.16	0.00	0.00	1.26	0.00	0.00	0.00	0.00	0.00
Cerambycidae sp.	**0.00**	**0.00**	**0.05**	**0.00**	**0.05**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.16	0.00	0.16	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coccinellidae sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elateridae sp.	**0.00**	**0.00**	**0.00**	**0.05**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.16	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Scarabaeidae sp.	**0.00**	**0.00**	**0.00**	**0.05**	**0.10**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.16	0.21	0.00	0.16	0.00	0.00	0.00	0.00	1.26	0.00	0.00	0.00	0.00
Staphylinidae sp.	**0.05**	**0.00**	**0.00**	**0.10**	**0.00**	**0.05**	**0.00**	**0.10**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.16	0.00	0.00	0.32	0.00	0.16	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tenebrionidae sp.	**0.15**	**0.65**	**0.30**	**0.25**	**0.10**	**0.20**	**0.25**	**0.20**	**0.00**	**0.00**	**0.00**	**0.40**	**0.00**	**0.40**	**0.00**	**0.00**
	0.24	0.67	0.42	0.35	0.21	0.48	0.26	0.26	0.00	0.00	0.00	1.26	0.00	1.26	0.00	0.00

.	Litter								Soil							
	Rainy				Dry				Rainy				Dry			
	B	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**
Dytiscidae sp.	**0.00**	**0.00**	**0.00**	**0.10**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Larva	**0.05**	**0.00**	**0.00**	**0.10**	**0.05**	**0.10**	**0.00**	**0.00**	**0.00**	**1.20**	**0.00**	**1.20**	**0.00**	**0.00**	**0.00**	**0.00**
	0.16	0.00	0.00	0.21	0.16	0.32	0.00	0.00	0.00	1.93	0.00	1.93	0.00	0.00	0.00	0.00
Termites (total)	**0.30**	**0.30**	**0.00**	**0.10**	**0.00**	**0.10**	**0.00**	**1.65**	**39.20**	**10.40**	**3.60**	**3.20**	**18.00**	**0.00**	**0.00**	**0.00**
	0.63	0.79	0.00	0.32	0.00	0.21	0.00	5.04	72.17	21.27	8.10	8.80	56.92	0.00	0.00	0.00
Ancistrotermes sp.	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**20.80**	**3.60**	**2.40**	**2.80**	**0.00**	**0.00**	**0.00**	**0.00**
	0.16	0.00	0.00	0.00	0.00	0.16	0.00	0.00	65.78	10.06	7.59	8.85	0.00	0.00	0.00	0.00
Macrotermes sp.	**0.25**	**0.30**	**0.00**	**0.10**	**0.00**	**0.05**	**0.00**	**1.65**	**18.40**	**6.80**	**1.20**	**0.40**	**18.00**	**0.00**	**0.00**	**0.00**
	0.63	0.79	0.00	0.32	0.00	0.16	0.00	5.04	41.62	20.14	3.79	1.26	56.92	0.00	0.00	0.00
Earthworms	**0.00**	**0.15**	**0.05**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.80**	**0.00**	**2.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.24	0.16	0.16	0.00	0.00	0.00	0.00	0.00	1.69	0.00	3.89	0.00	0.00	0.00	0.00
Chilopoda	**0.15**	**0.65**	**0.10**	**0.20**	**0.00**	**0.05**	**0.00**	**0.10**	**0.00**	**0.00**	**0.00**	**0.80**	**0.00**	**0.40**	**0.00**	**0.00**
	0.24	1.08	0.21	0.42	0.00	0.16	0.00	0.21	0.00	0.00	0.00	2.53	0.00	1.26	0.00	0.00
Diplopoda	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arachnida	**0.70**	**2.15**	**0.80**	**1.25**	**0.90**	**1.75**	**0.65**	**1.50**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.54	2.40	0.79	1.62	1.17	1.42	0.71	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

.	Litter								Soil							
	Rainy				Dry				Rainy				Dry			
	B	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**
Ants (total)	**1.30**	**3.05**	**1.80**	**3.15**	**0.75**	**4.05**	**0.75**	**1.80**	**0.00**	**0.40**	**0.40**	**1.20**	**34.40**	**0.00**	**0.00**	**0.00**
	1.09	2.11	1.48	6.31	0.72	6.10	0.49	3.12	0.00	1.26	1.26	2.70	108.78	0.00	0.00	0.00
Odontoponera	**0.25**	**1.05**	**0.95**	**0.45**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
transversa	0.35	1.30	0.69	0.50	0.00	0.00	0.00	0.00	0.00	0.00	1.26	0.00	0.00	0.00	0.00	0.00
Hypoponera sp. 1	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hypoponera sp. 2	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ectatomma sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odontomachus sp. 1	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odontomachus sp. 2	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odontomachus sp. 3	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pachycondyla sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.05**	**0.70**	**0.00**	**0.30**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.16	0.67	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Platythyrea sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.15**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

.	Litter								Soil							
	Rainy				Dry				Rainy				Dry			
	B	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**
Pheidole sp. 1	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pheidole sp. 2	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morphospecies sp. 1	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Morphospecies sp. 2	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.80**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.69	0.00	0.00	0.00	0.00
Morphospecies sp. 3	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Myrmecina sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calyptomyrmex sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**34.40**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	108.78	0.00	0.00	0.00
Paratrechina	**0.20**	**0.00**	**0.05**	**1.90**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
longicornis	0.63	0.00	0.16	6.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anoploepis	**0.00**	**0.00**	**0.00**	**0.05**	**0.10**	**0.00**	**0.15**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
gracilipes	0.00	0.00	0.00	0.16	0.21	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oecophylla	**0.35**	**0.65**	**0.15**	**0.45**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
longinoda	0.82	1.27	0.34	0.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

.	Litter								Soil							
	Rainy				Dry				Rainy				Dry			
	B	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**	**B**	**BH**	**H**	**HH**
Camponotus sp. 1	**0.15**	**0.15**	**0.00**	**0.05**	**0.10**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.34	0.34	0.00	0.16	0.21	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 2	**0.15**	**0.05**	**0.05**	**0.00**	**0.45**	**0.35**	**0.60**	**0.35**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.47	0.16	0.16	0.00	0.64	0.67	0.39	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 3	**0.05**	**0.20**	**0.20**	**0.10**	**0.00**	**1.90**	**0.00**	**0.85**	**0.00**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.16	0.26	0.35	0.21	0.00	3.92	0.00	2.52	0.00	1.26	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 4	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 5	**0.15**	**0.90**	**0.30**	**0.15**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.47	1.81	0.95	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 6	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Larva	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.55**	**0.00**	**0.25**	**0.00**	**0.00**	**0.00**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	1.74	0.00	0.79	0.00	0.00	0.00	1.26	0.00	0.00	0.00	0.00
Others	**0.40**	**0.80**	**0.15**	**0.95**	**6.15**	**10.55**	**5.15**	**7.55**	**0.00**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.70	0.86	0.34	0.90	5.05	5.09	4.89	4.08	0.00	1.26	0.00	0.00	0.00	0.00	0.00	0.00
Total	**3.15**	**7.80**	**3.25**	**6.55**	**8.25**	**17.05**	**6.90**	**12.95**	**39.20**	**13.20**	**4.40**	**9.20**	**52.40**	**0.80**	**0.00**	**0.00**
	1.72	5.64	1.92	6.22	6.49	9.66	5.34	6.89	72.17	20.83	8.10	10.84	117.04	2.53	0.00	0.00
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Appendix 2 Density of the macrofauna (mean and standard deviation, individual m^{-2}) in *Hevea* plantations with different soil organic managements; no organic input (S1), external organic input (S2) and *Pueraria* cover crop + external organic input (S3) during rainy period of 2006 and dry period of 2008.

	Litter						**Soil**					
	Rainy			**Dry**			**Rainy**				**Dry**	
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Coleoptera (total)	**1.20**	**0.58**	**0.78**	**0.35**	**0.31**	**0.36**	**2.00**	**0.90**	**1.30**	**0.00**	**0.80**	**1.40**
	0.63	0.71	0.80	0.34	0.72	0.68	2.83	1.69	2.92	0.00	1.62	1.93
Carabidae sp.	**0.20**	**0.00**	**0.01**	**0.05**	**0.04**	**0.04**	**0.00**	**0.00**	**0.10**	**0.00**	**0.20**	**0.20**
	0.35	0.00	0.08	0.16	0.17	0.13	0.00	0.00	0.63	0.00	0.88	0.88
Cerambycidae sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00
Coccinellidae sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**	**0.00**
	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.63	0.00
Elateridae sp.	**0.05**	**0.01**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.16	0.08	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Scarabaeidae sp.	**0.05**	**0.01**	**0.01**	**0.00**	**0.00**	**0.01**	**0.40**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**
	0.16	0.08	0.08	0.00	0.00	0.08	1.26	0.00	0.00	0.00	0.00	0.63
Staphylinidae sp.	**0.10**	**0.00**	**0.00**	**0.10**	**0.00**	**0.00**	**0.00**	**0.10**	**0.20**	**0.00**	**0.10**	**0.00**
	0.32	0.00	0.00	0.21	0.00	0.00	0.00	0.63	0.88	0.00	0.63	0.00
Tenebrionidae sp.	**0.25**	**0.29**	**0.38**	**0.20**	**0.26**	**0.25**	**0.40**	**0.30**	**0.30**	**0.00**	**0.20**	**1.10**
	0.35	0.53	0.54	0.26	0.68	0.52	1.26	1.07	1.07	0.00	0.88	1.81

	Litter						Soil					
	Rainy			Dry			Rainy				Dry	
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Dytiscidae sp.	**0.10**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.21	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00
Larva	**0.10**	**0.08**	**0.14**	**0.00**	**0.00**	**0.00**	**1.20**	**0.50**	**0.70**	**0.00**	**0.20**	**0.00**
	0.21	0.18	0.28	0.00	0.00	0.00	1.93	1.34	2.00	0.00	0.88	0.00
Termites (total)	**0.10**	**0.25**	**0.40**	**1.65**	**0.49**	**0.18**	**3.20**	**17.80**	**18.00**	**0.00**	**3.90**	**1.50**
	0.32	0.71	1.15	5.04	1.56	0.50	8.80	51.77	62.44	0.00	12.17	9.49
Ancistrotermes sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.03**	**2.80**	**0.30**	**9.60**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.11	8.85	1.90	55.67	0.00	0.00	0.00
Macrotermes sp.	**0.10**	**0.19**	**0.33**	**1.65**	**0.49**	**0.15**	**0.40**	**17.50**	**8.40**	**0.00**	**3.90**	**1.50**
	0.32	0.57	1.04	5.04	1.56	0.50	1.26	51.84	31.07	0.00	12.17	9.49
Earthworms	**0.10**	**0.03**	**0.05**	**0.00**	**0.00**	**0.00**	**2.00**	**0.60**	**0.00**	**0.00**	**0.20**	**0.10**
	0.32	0.16	0.22	0.00	0.00	0.00	3.89	1.71	0.00	0.00	0.88	0.63
Chilopoda	**0.20**	**0.13**	**0.18**	**0.10**	**0.04**	**0.05**	**0.80**	**0.20**	**0.00**	**0.00**	**2.20**	**1.80**
	0.42	0.33	0.55	0.21	0.13	0.15	2.53	0.88	0.00	0.00	3.50	2.39
Diplopoda	**0.00**	**0.03**	**0.15**	**0.00**	**0.00**	**0.00**	**0.00**	**0.20**	**0.00**	**0.00**	**0.00**	**1.50**
	0.00	0.16	0.48	0.00	0.00	0.00	0.00	1.26	0.00	0.00	0.00	8.24
Arachnida	**1.50**	**1.25**	**1.10**	**1.50**	**1.06**	**0.58**	**0.00**	**1.20**	**1.00**	**0.00**	**1.10**	**1.10**
	1.72	1.01	1.01	1.33	0.93	0.68	0.00	2.59	1.75	0.00	2.02	2.22
Ants (total)	**3.30**	**3.43**	**2.50**	**1.80**	**2.86**	**1.68**	**1.20**	**4.80**	**16.20**	**0.00**	**4.00**	**2.80**
	6.25	2.27	3.04	3.12	3.97	2.98	2.70	9.93	69.29	0.00	13.19	8.06

	Litter						Soil					
	Rainy			Dry			Rainy				Dry	
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Odontoponera	**0.45**	**0.34**	**0.20**	**0.00**	**0.00**	**0.00**	**0.00**	**0.60**	**0.30**	**0.00**	**0.00**	**0.00**
transversa	0.50	0.50	0.34	0.00	0.00	0.00	0.00	1.71	1.90	0.00	0.00	0.00
Hypoponera sp. 1	**0.00**	**0.13**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**1.60**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.29	0.08	0.00	0.00	0.00	0.00	6.20	0.00	0.00	0.00	0.00
Hypoponera sp. 2	**0.00**	**0.03**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**	**0.10**	**0.00**	**0.00**	**0.00**
	0.00	0.11	0.08	0.00	0.00	0.00	0.00	0.63	0.63	0.00	0.00	0.00
Ectatomma sp.	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odontomachus sp. 1	**0.00**	**0.34**	**0.09**	**0.00**	**0.20**	**0.03**	**0.00**	**0.20**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	1.29	0.41	0.00	1.26	0.16	0.00	0.88	0.00	0.00	0.00	0.00
Odontomachus sp. 2	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odontomachus sp. 3	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00
Pachycondyla sp.	**0.00**	**0.00**	**0.00**	**0.30**	**0.50**	**0.36**	**0.00**	**0.00**	**0.00**	**0.00**	**1.60**	**0.50**
	0.00	0.00	0.00	0.42	0.95	0.44	0.00	0.00	0.00	0.00	8.87	1.62
Platythyrea sp.	**0.00**	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pheidole sp. 1	**0.00**	**0.00**	**0.38**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	2.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

	Litter						Soil					
	Rainy			Dry			Rainy			Dry		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Morphospecies sp. 1	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**2.70**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.08	0.00	0.00	0.00
Morphospecies sp. 2	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.80**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.00	0.00	0.00	0.00	1.69	0.00	0.00	0.00	0.00	0.00
Morphospecies sp. 3	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Calyptomyrmex sp.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.70**	**0.60**
	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	4.43	3.79
Paratrechina	**1.90**	**0.15**	**0.31**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**3.30**	**0.00**	**0.00**	**0.00**
longicornis	6.01	0.95	1.12	0.00	0.00	0.00	0.00	0.00	20.87	0.00	0.00	0.00
Anoploepis	**0.05**	**0.16**	**0.39**	**0.00**	**0.55**	**0.24**	**0.00**	**0.70**	**0.10**	**0.00**	**0.20**	**0.40**
gracilipes	0.16	0.49	1.16	0.00	2.93	1.42	0.00	4.43	0.63	0.00	1.26	1.98
Oecophylla	**0.45**	**1.11**	**0.34**	**0.00**	**0.35**	**0.18**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
longinoda	0.72	1.25	0.80	0.00	0.75	0.55	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 1	**0.05**	**0.04**	**0.15**	**0.00**	**0.14**	**0.11**	**0.00**	**0.10**	**0.00**	**0.00**	**0.20**	**0.50**
	0.16	0.13	0.50	0.00	0.60	0.52	0.00	0.63	0.00	0.00	1.26	2.25
Camponotus sp. 2	**0.00**	**0.03**	**0.01**	**0.35**	**0.21**	**0.45**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.10**
	0.00	0.11	0.08	0.58	0.47	1.83	0.00	0.00	0.00	0.00	1.07	0.63
Camponotus sp. 3	**0.10**	**0.43**	**0.18**	**0.85**	**0.55**	**0.21**	**0.00**	**0.40**	**0.00**	**0.00**	**1.00**	**0.70**
	0.21	0.97	0.43	2.52	1.13	0.62	0.00	1.98	0.00	0.00	3.36	3.38

	Litter						Soil					
	Rainy			Dry			Rainy				Dry	
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Camponotus sp. 4	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 5	**0.15**	**0.26**	**0.16**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.34	0.75	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Camponotus sp. 6	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
larva	**0.00**	**0.09**	**0.04**	**0.25**	**0.36**	**0.09**	**0.40**	**1.00**	**9.70**	**0.00**	**0.00**	**0.00**
	0.00	0.30	0.24	0.79	1.65	0.48	1.26	3.59	43.22	0.00	0.00	0.00
Others	**1.20**	**0.43**	**0.73**	**7.55**	**1.63**	**1.49**	**0.00**	**0.50**	**0.60**	**0.00**	**0.00**	**0.50**
	0.92	0.68	3.05	4.08	1.32	1.02	0.00	2.59	1.93	0.00	0.00	1.62
Total	**6.80**	**5.38**	**5.15**	**12.95**	**6.39**	**4.33**	**9.20**	**26.20**	**37.10**	**0.00**	**12.20**	**10.70**
	6.14	2.48	3.85	6.89	4.78	3.35	10.84	56.82	91.69	0.00	17.73	14.19
N	10	40	40	10	40	40	10	40	40	10	40	40