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RESEARCH ARTICLE

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Ecological process of leaf litter decomposition in tropical rainforest in Xishuangbanna, southwest China. III. Enzyme dynamics

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Abstract We tested the dynamics of nine enzymes during leaf litter decomposition in Xishuangbanna tropical rainforest both in the field and laboratory to explore the response of enzyme dynamics to decomposition under different food-web structures. We used coarse and fine (1 mm and 100 μm mesh size, respectively) litterbags in the field to create different food-web structures during litter decomposition. Most soil macrofauna such as nematodes could access only the coarse mesh litterbags, leaving only microbiota, such as mites, in the fine mesh litterbags. In the laboratory, sterilization and inoculation were adopted to investigate different enzyme dynamics with nematodes or only microbiota participating in litter decomposition. Invertase and amylase increased more for shorter food webs at the early stages of decomposition, while activities of endocellulase, β-glucosidase, xylanase and polyphenoloxydase increased to their maxima at the later stages, but greater increase occurred with extended food webs. Invertase and amylase had negative relationships and endocellulase, β-glucosidase, xylanase and polyphenoloxydase had positive relationships with litter decomposition (mass loss). The activities of enzymes responded to the process of litter decomposition. Invertase and amylase played key roles for microbiota utilizing the substrates at early stages of decomposition, while endocellulase, β-glucosidase, xylanase and polyphenoloxydase worked on the further decay of recalcitrant compounds at later stages. All enzymes related to carbon decay acted as effective indicators of litter decomposition. The decomposition of plant organic matter was essentially an enzymatic process.

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1 Introduction

Leaf litter is one of the most important sources of organic matter for soils in the forest ecological systems (Ke et al., 1999). Leaves will decompose soon after they drop (Takeda, 1988). As the initial decomposers, microbes play an irreplaceable role in this process. Microbes decompose organic remnants through excreting extra-cellular and intra-cellular enzymes. Extra-cellular processes mainly decompose amyloses, including fibrin, xylogen, amylum and sucrose. These carbohydrates are the most significant components in organic remnants of higher plants and most plant species and their decomposition have an important effect on the carbon cycle in the natural biosphere (Kshattriya et al., 1992). Research on changes in these enzymes activity helps us further to understand the mechanism of organic matter decomposition (Burns, 1978; Sinsabaugh et al., 1991; Kandeler et al., 1999; Luxhi et al., 2002).

Generally, the decomposition rates of leaf litter in tropical and subtropical broad-leaved forests and pine trees are from 1.0 to 1.75 (Brown and Lugo, 1982; Cuevas et al., 1991). For example, the turnover rate (time required to decompose 95%) of leaf litter decomposition is 1.7 to 3 years. It requires just 1.5 years in the Xishuangbanna seasonal tropical rain forest ecosystem in China (Ren et al., 1999), compared to those needing 3 to 5 years (Zhang et al., 1999) or even 8 to 14 years (Wang and Huang, 2001) in temperate deciduous broad-leaved forests. It is much faster in Xishuangbanna. The differences in turnover rates may be affected by the environment (soil and climate) and are mainly due to biological actions.

How do microbes function in such a rapid turnover ecosystem? Microbes are the most direct factors in the decomposition of organic material and enzyme activity is an indicator for microbial biomass and activity (Xu and

Zheng, 1986). The question arises then, whether the intensity of enzyme activity can be used as an effective measure for the decomposition process. If, on the other hand, the decomposition process is constantly changing, as it is affected by a variety of factors, then the next question posed is: "Does a certain response exist between the dynamic change in the decomposition process and enzyme activity dynamics?" In order to understand the relationship between the process of organic matter decomposition and enzyme activity, different decomposition processes controlled by biological and non-biological factors were designed in our study. Changes of nine kinds of enzyme activities, i.e., exoglucanase, endocellulase, β-glucosidase, xylanase, polyphenoloxydase, amylase, invertase, acid phosphatase and alkali phosphatase, during different leaf litter decomposition periods under various affecting factors, were measured and compared. The relationships between the change of enzyme activity and the dynamics of microbiology as well as decomposition processes were analyzed and the intensity of enzyme activity as an index of organic matter decomposition process was verified. Studies on these aspects are rarely seen in China.

2 General situation of study sites

Our study area was located at the Tropical Ecological Station of the Chinese Academy of Sciences (in Menglun, Mengla County, Xishuangbanna, Yunnan Province, 21°56'N, 101°16'E). According to the data of many years from the tropical weather station of the Chinese Academy of Sciences, the annual rainfall in the study site is about 1600 mm, the average temperature is 21.4–22.6°C with little changes during the entire year. Controlled by tropical monsoons, the study area has clear dry and wet seasons. About 83% of annual rainfall occurs in the wet season (May to October) and just 17% in the dry season (November to April of the following year). The red soil developed from cretaceous sandstone, usually more than 80 cm thick. The soil surface is covered with a layer of about 2–3 cm deadwood and leaf litter. The soil is thick, loose, porous and permeable and has a good water hold capacity with high soil moisture during the wet season. The average monthly temperature, rainfall at sampling time (April 2004 to March 2005) and basic soil physical and chemical properties (14 randomly sampled points) in the study sites are shown in Tables 1 and 2.

Sample sites were located at the Nature Reserve at 600 m elevation. About 1300 plant species were distributed in this area. *Pometia tomentosa*, *Terminalia myriocarpa*, *Myristica yunnanensis*, *Horsfieldia tetratepala*, *Homalium laoticum* and *Barringtonia macrostachya* are the dominant tree species. The tree height of the community is 48 m and the forest has clear structures and layers, at an age of 200 years. Besides, there were also a great number of shrubs and herbaceous plants, an abundance of vines and saprophytic plants and the sites are notable for their board-root and stem-floor phenomena (Zhang et al., 1994; Cao et al., 1996; Yang, 2004).

3 Material and methods

3.1 Field study

Litterbags were flatly placed on the soil surface in the field. A given amount of fresh leaf litter (of which *P. tomentosa* accounted for 45%) was collected around the study site in March, 2004 (the best leaf collection period from deciduous trees), naturally air-dried indoors and then cut into 2 cm×5 cm pieces. After that, impurities were removed through a 5 mm-diameter sieve. The mixed samples (for the basic physio-chemical properties of mixed leaf litter see Table 3) were then separately enclosed into two kinds of different specifications of numbered mesh litterbags, where mesh sizes were, respectively, 1 mm and 100 µm. Since the width of the nematodes is generally 3–100 µm, while that of acarids is between 100 µm and 2 mm, the mesh size of 100 µm restricted access of acarids, while nematodes had free access and the 1 mm mesh permitted the entry of most acarids, i.e., two processes were conducted simultaneously. Litterbag specifications were 20 cm×20 cm and 20 g air-dried samples (converted into 19.1 g of dry matter) were loaded in each bag, four replications for each sample.

The decomposition mesh litterbags were placed flatly on the surface of the forest floor, after the removal of the original litter with a flat spade, along contour lines (elevation 650 m) and 5 m from top to bottom (shaded

Table 1 Average monthly temperature and amount of monthly precipitation at sampling time (April 2004 to March 2005) in Xishuangbanna

item	2004									2005		
	Apr.	May	Jun.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
temperature/°C	21.5	23.6	24.7	25.5	25.2	25.4	24.2	22.3	19.8	15.0	16.8	19.3
precipitation/mm	263.0	779.0	223.9	79.9	169.0	204.6	160.1	56.8	45.8	0.0	1.5	0.0

Table 2 Physical and chemical properties of the top soil (0–2.5 cm) in the experiment fields in Xishuangbanna (mean ± *SE*, *n* = 14)

organic matter /(g·kg^{-1})	total nitrogen /(g·kg^{-1})	C/N	pH	density /(10^9 mg·m^{-3})
48.3 ± 0.8	2.3 ± 0.0	12.3 ± 1.2	4.75 ± 0.22	1.02 ± 0.10

side), simulating natural conditions, on April 27, 2004. The mesh bags were entirely in contact with the surface soil. Bags were placed at 5 m intervals with a disposal length of about 100 m. Sampling was conducted randomly in the disposal section, respectively, on May 27, July 17, September 12, November 6 in 2004 and January 9 and March 13 in 2005. The collected mesh litterbags were then brought back to the laboratory. The samples were treated immediately and enzyme activity was measured. The entire experimental period lasted 310 d.

3.2 Simulated laboratory study

Surface soil from 0–2.5 cm beneath the forest floor in the seasonal, tropical rain forest of Xishuangbanna (the same place as the field sites) was sampled for a simulated laboratory study. Ten pieces of sample area (1 m×1 m) with well-proportioned vegetation were selected, fresh surface soil was sampled after scratching the surface litter, then plant roots were selected and large amounts of sand and debris were removed with a 1 mm-sieve from the samples. Organic matter content, full nitrogen content, C/N ratio and the pH value of the treated soil were respectively 4.26%, 0.21%, 12.0%, and 4.71%. The material was decomposed using a method similar to that for field studies, cut into 0.5 cm×1 cm pieces and naturally air-dried. Debris were removed with a 2 mm-sieve. The material was then mixed evenly and loaded into 10 cm×10 cm mesh litterbags (100 μm mesh), at 6 g (converting to dry matter of 5.81 g) for each bag.

Two treatments were assigned: 1) sterilized soil inoculated with microbiota + mixed leaf litter (M) and; 2) sterilized soil inoculated with microbiota + nematodes + mixed leaf litter (MN). The process for sterilizing and vaccinating decomposed material and cultivation soil (Setala et al., 1988) were as follows: the material was first dried for 48 h at 80°C and then frozen for 24 h at −80°C to kill soil animals and other kinds of dormant life, such as sporangium. Sterilized soil was sprayed with a bacterial suspension (dissolving 25 g fresh soil into 0.5 L sterile distilled water, mixing round, then removing all kinds of soil animals and their eggs and sporangium, respectively, with a 38 μm- and 10 μm-sieve) to vaccinate the micro-organisms. Until the soil moisture content reached 70%, the mixture was, respectively, loaded into 50 cm×50 cm×7 cm plastic cultivation basins with 5 cm thick soil for each basin. These were then pre-cultivated for 30 d in an artificial climate box (with relative humidity of 70%, temperature of 24 ± 0.5°C, and daily light intensity of 1000 lx for 12 h).

Inoculation methods for soil nematodes were as follows: nematodes were separated by the wet funnel method. Other soil animals (*Collembola*, *Rotifer*, *Enchytraeid*, a small number of *Mites*, etc.) were removed by microscopic examination (10×4 times) for the vaccination. About 300 nematodes, including 200 fresh bacterial nematodes, 15 fresh fungal nematodes, 80 phytophagous nematodes and 5 predatory nematodes were inoculated into every 100 g of dry soil.

The sterilized mesh litterbags were placed flatly onto the cultivation soil, 25 bags for each basin. Then, the basins were sealed with plastic sheeting (with four pieces of 2 cm ×2 cm ventilation stoma made with a 0.2 μm porous membrane, to prevent the entry of other bacteria) and placed in the artificial climate box (with a relative humidity of 70%, temperature of 22 ± 0.5°C and daily light intensity of 1000 lx for 12 h) for cultivation. Regular samplings were made for measurements and the water level was maintained after each sampling. The sampling scheme was designed for six sampling intervals as follows: 20, 20, 20, 20, 30 and 30 d, each with three replications (two bags of samples for each repeat). The experimental period was 140 d. After sampling in the field, mesh bags were removed carefully and plant roots, stems and sand in the samples were also removed. Then, the samples were washed several times with distilled water to remove attached impurities (soil and sand). The samples were sieved with a 1 mm nylon mesh filter and then cut into pieces < 5 mm after removing the water, and then sealed into pockets for preservation at 4°C. Enzyme activity was measured within one week (some samples were preserved at −20°C for longer period for measurements). Laboratory samples were directly mixed and cut into pieces < 0.5 mm for extraction of crude enzymes.

3.3 Preparation for crude enzymes

Amounts of 1.00 g samples were weighed and put into an ice bath mortar. Adding about 4 mL pre-cooled (4°C, 12 h) extraction (1 mol/L $CaCl_2$ solution, which contains 0.5 mL soil temperature 80°C), the homogenate was ground rapidly (particles < 0.5 mm), then transferred to a 50 mL centrifuge tube, by adding 2 g pre-cooled PVPP (XLPE Pyrrolidone, adding 10% HCl, boiled for 10 min., followed, by using NaOH to counteract it and then using distilled water to wash it several times before use, preserving at 4°C), it was oscillated (120 r/min) with an oscillator at low-temperature (4°C) for 1 h (or staying overnight after shaking) and then frozen and centrifugated for 20 min at 3000 r/min. The clear upper part of the liquid

Table 3 Basic properties of material (mixed leaf litter) used in the study (mean ± *SE* , *n* = 3)

C/%	N/%	C/N ratio	ash/%	cellulose/%	lignin/%
49.1 ± 0.22	1.11 ± 0.01	44.3 ± 0.16	7.41 ± 0.02	41.29 ± 0.06	28.26 ± 0.04

Note: Values are percentages based on ash-free dry mass.

was filtrated, respectively, with 2.7 and 1.2 mm Whatman filter paper and the filtrate was transferred into dialysis bags (interception molecular weight of 10 kD) and dialyzed at 4°C for 48 h in 2 mmol/L of a Bis-Tris buffer (pH 6.0), replacing the dialysis fluid every 12 h during this process. After that, the crude enzyme solution was transferred into a test tube with 25 mL capacity to a constant volume and preserved at 4°C for use. Another part of the samples was also ground, repeating the above operation. After the dialysis, PEG (polyethylene glycol, molecular weight of 15–20 kD) bags were covered outside the dialysis bags to contract the crude enzyme liquid to 1/5 to 1/10 of the original volume for determination of polyphenol activity (Criquet et al., 1999). The detailed crude enzyme extraction process is shown in Fig. 1.

3.4 Measurement of enzyme activity

The following method was used in measuring enzyme activity including that of exoglucanase, endocellulase, *β*-glucosidase, xylanase, amylase and invertase, using multi-carbohydrate material for the substrate: 0.6 mL substrate (respectively, 0.7% CMC (USA Sigma Corporation), 10 mg absorbent cotton, 0.7% *Salicylaldehyde glycoside* (USA Sigma Corporation), 1.2% Xylan (USA Sigma Corporation), 0.6% soluble starch, a 0.6% sucrose acetate buffer (pH 5.5, 0.2 mol/L) was added into 0.4 mL of crude enzyme liquid. Then, the mixture was put into a water bath at 50°C for 24 h (starch and invertase reacting for 3 h). Two contrasts of adding deactivated enzyme and without substrate were established, each were repeated twice. The quantity of the reducing sugar was measured with a 3, 5-dinitrosalicylic acid (DNS) deoxidization method (red-brown amide were created in the reaction between reducing sugar or glucose with 3, 5-dinitrosalicylic acid). The color change (product concentration) was positively related with the amount of reducing sugar or glucose and was thus positively related to enzyme activity in the enzyme sample.

After this reaction, 0.5 mL 3, 5-dinitrosalicylic acid reagents (dissolving 6.3 g DNS with a small amount of water, then successively adding a 2 mol/L NaOH solution of 262 mL, a 500 mL hot solution containing 185 g kalium-sodium tartrate, 5 g crystalline phenol and 5 g sodium sulfite, stirring to dissolve; after that, adding distilled water after the solution cooling to fill a constant volume of 1000 mL, storing the solution in a brown bottle, for use after one week) were added to terminate the reaction. The solution was put in a boiling water bath for 5 min and filled to a constant volume of 15 mL after cooling by coloring (752-spectrophotometer) in 540 nm (575 nm for the maltose). The amount of reducing sugar production can be determined from the reducing sugar or glucose standard curve that checks for absorbance values; the units for enzyme vitality were μmol glucose/(g AFDM·h).

Polyphenol oxidase: crude enzymes were concentrated 5–10 times by the PEG (polyethylene glycol, molecular weight of 20 kD, USA Sigma Corporation) to determine polyphenol oxidase. We added a 1.0 mL 25 mmol/L pyrogallol solution into a 1.0 mL concentrated enzyme solution. A small amount of ether was injected into the solution after cultivation at 30°C for 30 min, generating a purple gallic which could be extracted from the ether. The extraction was repeated several times until the ether was colorless. Ether extracts were mixed and filled to a constant volume of 15 mL, colored in 430 nm. Two contrasts of adding deactivated enzymes and without substrate were established and each were repeated twice. The unit for enzyme vitality was mg purple gallic/(g AFDM·h).

Acid/ALP phosphatase: a 0.6 mL substrate (benzene disodium phosphate, Sigma Corporation) buffer (respectively

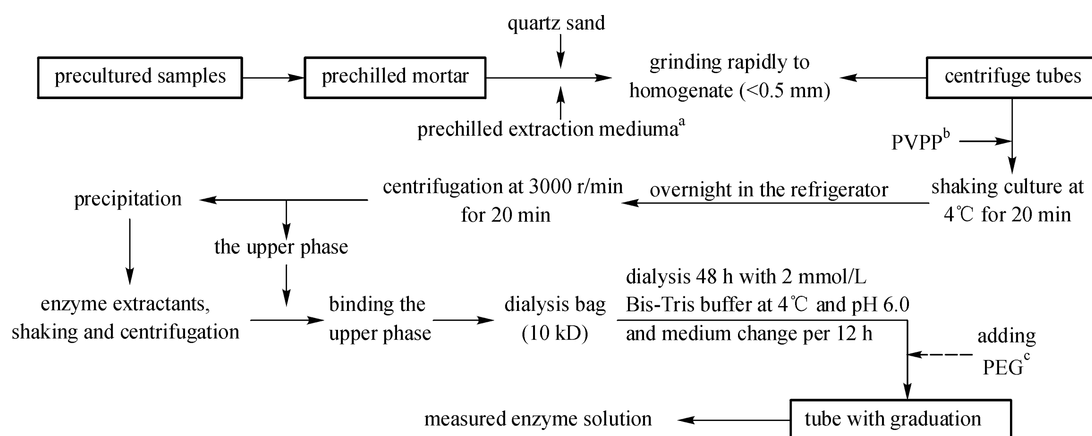

Fig. 1 Flow chart for enzyme extraction from plant material. Note: a: extraction solvent was made in addition of $CaCl_2$ 110.99 g/L and Tween 80 0.5 mL/L. b: polyvinylpolypyrrolidone, used as decoloring agent. c: polyethylene glycol, molecular mass 15–20 kD, used to concentrate the dialyzed extract.

50 mmol/L, pH 5.0 of an acetate buffer and 50 mmol/L, pH 8.5 of a Tris-HCl buffer) was added into 0.4 mL of a crude enzyme solution, after which the mixture was put into water bath at 37°C for 3 h. The reaction was terminated by adding a 0.5 mL boracic acid buffer (50 mmol/L, pH 9.6). Then, 0.2 mL Gibb reagents (6.68 mmol/L 2,6-2bromophenyl quinone Polyimide chlorine solution, Sigma Corporation) were added for the color display for 20 min, then filled to a constant volume of 15 mL, coloring in 578 nm. Two contrasts of adding deactivated enzyme and without substrate were established, each were repeated twice. The unit for enzyme vitality was mmol hydroxybenzene/(g AFDM·h).

4 Results and analysis

4.1 Changes of enzyme activity involved in decomposition of simple compounds

The results of our field study show that during the process of leaf litter decomposition, invertase activity (Fig. 2) increased rapidly to its maximum value at an early stage (0–80 d) and then declined rapidly. After decomposing for 310 d, enzyme activity almost fell to 0 (1.2 μmol glucose/(g AFDM·h)). Invertase activity of C (coarse mesh) treatment was significantly higher than that of F (fine mesh) treatment ($p < 0.05$).

The result in our laboratory study was similar to that from the field. Invertase activity also increased rapidly (the maximum value was 30.9 μmol glucose/(g AFDM·h)) at an early stage of decomposition (0–40 d), then declined rapidly. Invertase activity of vaccinated nematode treatment (MN) increased slowly and only slightly (11.1 μmol glucose/(g AFDM·h)) at the later stage of decomposition (110–140 d). Invertase activity of vaccinated nematode treatment was significantly higher than that of the unvaccinated nematode treatment (M treatment) ($p < 0.05$).

Compared with changes in invertase dynamics, changes of amylase activity were more complex (Fig. 3). We found in our field study that amylase activity declined substantially at the middle and later stages during decomposition and maintained a rate of 10–20 μmol glucose/(g AFDM·h) at other stages. Activity of C treatment amylase was significantly higher than that in the F treatment at a later stage of decomposition ($p < 0.05$). But in the laboratory study, amylase dynamics with different treatments varied greatly. Amylase activity without vaccinated nematodes (M) treatment always maintained a high level (60–80 μmol glucose/(g AFDM·h)) at the early and middle stages of decomposition and dropped rapidly to 20 μmol glucose/(g AFDM·h) at a later stage. Amylase activity with MN treatment was generally maintained at about 20 μmol glucose/(g AFDM·h), except that it increased slightly (46 μmol glucose/(g AFDM·h)) in the second sample (40 d). During the entire experimental period, amylase

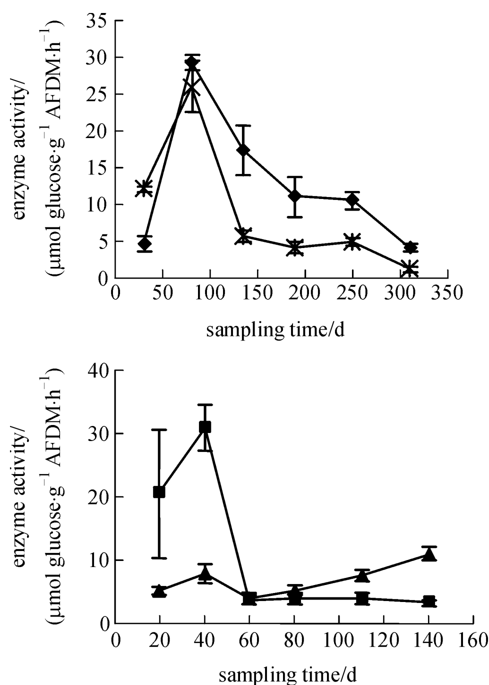

Fig. 2 Invertase activity during decomposition of leaf litter. Bars are means with standard error ($n=4$ for field and $n=3$ for inoculated study). Note: ◆ C: coarse mesh; *F: fine mesh; ■ M: incubated microbiota only; ▲MN: incubated microbiota and nematodes. The same comments apply to following figures.

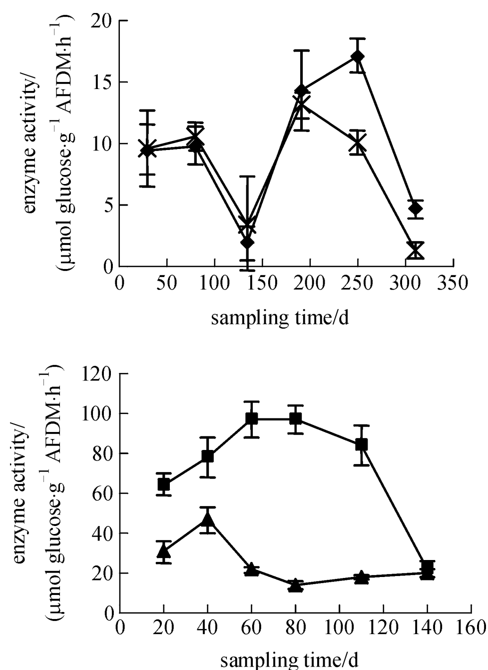

Fig. 3 Amylase activity during decomposition of leaf litters. Bars are means with standard error ($n=4$ for field and $n=3$ for inoculated study).

activity in M treatment was significantly ($p < 0.01$) higher than that in MN treatment.

Activity of acid phosphatase was significantly ($p < 0.01$) higher than that of alkaline phosphatase (Fig. 4) in all treatments. The activity of acid phosphatase increased initially (0–135 d), then decreased (135–310 d) in the field study, but the vitality was under 20 μmol phenol/(g AFDM·h), while acid phosphatase showed an increasing trend in the laboratory study, with a general activity above 20 μmol phenol/(g AFDM·h), far higher than that in the field. Alkaline phosphatase had lower activity, generally increased at first and then declined gradually.

4.2 Changes of enzyme activity involved in decomposition of recalcitrant compounds

Activity of endoglucanase, β-glucosidase and xylanase all show an increasing trend in the field study (Fig. 5) and arrived at their maxima at the middle and later stages during decomposition. The activity of phenol oxidase maintained a higher level at the early and later stages and declined at the middle stage during decomposition. Endoglucanase with C treatment, β-glucosidase and xylanase are all more active than that of F treatment ($p < 0.05$). Exoglucanase (C_1 enzyme) differed with other enzymes and they were most active at the initial stage of decomposition, which gradually declined during the decomposition process. There were no differences among treatments.

Activity of endoglucanase, β-glucosidase, xylanase and phenol oxidase increased rapidly (Fig. 6) at the initial stage of litter decomposition and then changed slowly to a constant value in the laboratory study. Endoglucanase with MN treatment and xylanase were more active than in the M treatment ($p < 0.05$). Exoglucanase activity declined all the time along the decomposition process until it was below 2 μmol glucose/(g AFDM·h) at the end of the process.

5 Discussion

5.1 Dynamic response of enzyme activity to microbe and decomposition processes

Invertase and amylase played important roles in the decomposition of organic residue in the field, by participating in the transformation and decomposition of carbon compounds. Some researchers have thought that amylase may mainly come from the secretion of micro-organisms and that the invertase enzyme may be partly from the plant itself (Ross and Roberts, 1973). With the process of decomposition, invertase activity gradually weakened and amylase activity remained relatively stable. This phenomenon shows that the size of amylase was very important for microbes to decompose and use a carbon source at the initial decomposition stage. In most stages of decomposition, microbes made further use of simple carbon compounds, mainly through the secretion of microbial amylase.

Amylase activity with different treatments varied greatly both in the field and the laboratory, which suggests that microorganisms mainly make further use of substrates in separate decompositions (M treatment) through secreting this enzyme in the absence of environmental and predation pressure. When they are suffering environmental and predation pressure, vaccinated nematodes (MN treatment) or micro-organisms accelerate the decomposition process and microbial populations fluctuate (Zhang et al., 2007). At the same time, the substrate utilization style of microbes also changed, such as that of endoglucanase C_x, β-glucosidase activity, clearly increased, i.e., microbes decompose polysaccharide and at the same time further hydrolyze oligosaccharides into glucose for use in β-glucosidase. Therefore, dynamic responses exist in these enzyme activities with microbial activity and in the decomposition processes. Phosphatase is induced and synthesized by microbes when phosphorus conditions are lacking. Organic residues, particularly tree leaf litter,

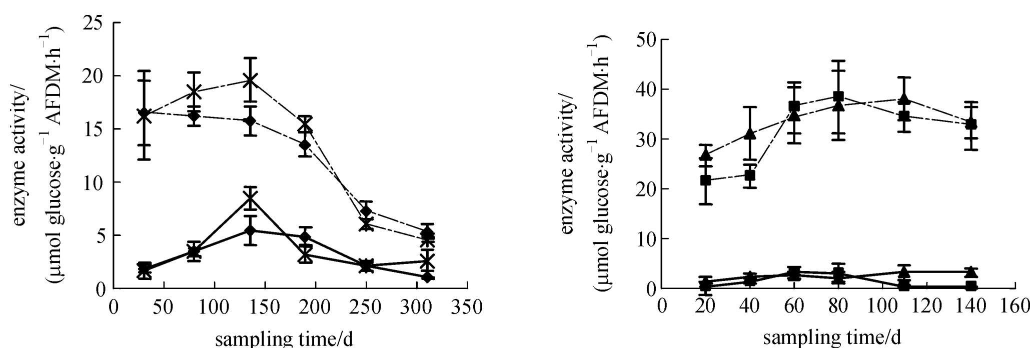

Fig. 4 Acid and alkaline phosphatase activities during decomposition of leaf litter in field ($n = 4$) and simulated ($n = 3$) study. Note: ——— acid , —·—·— alkaline.

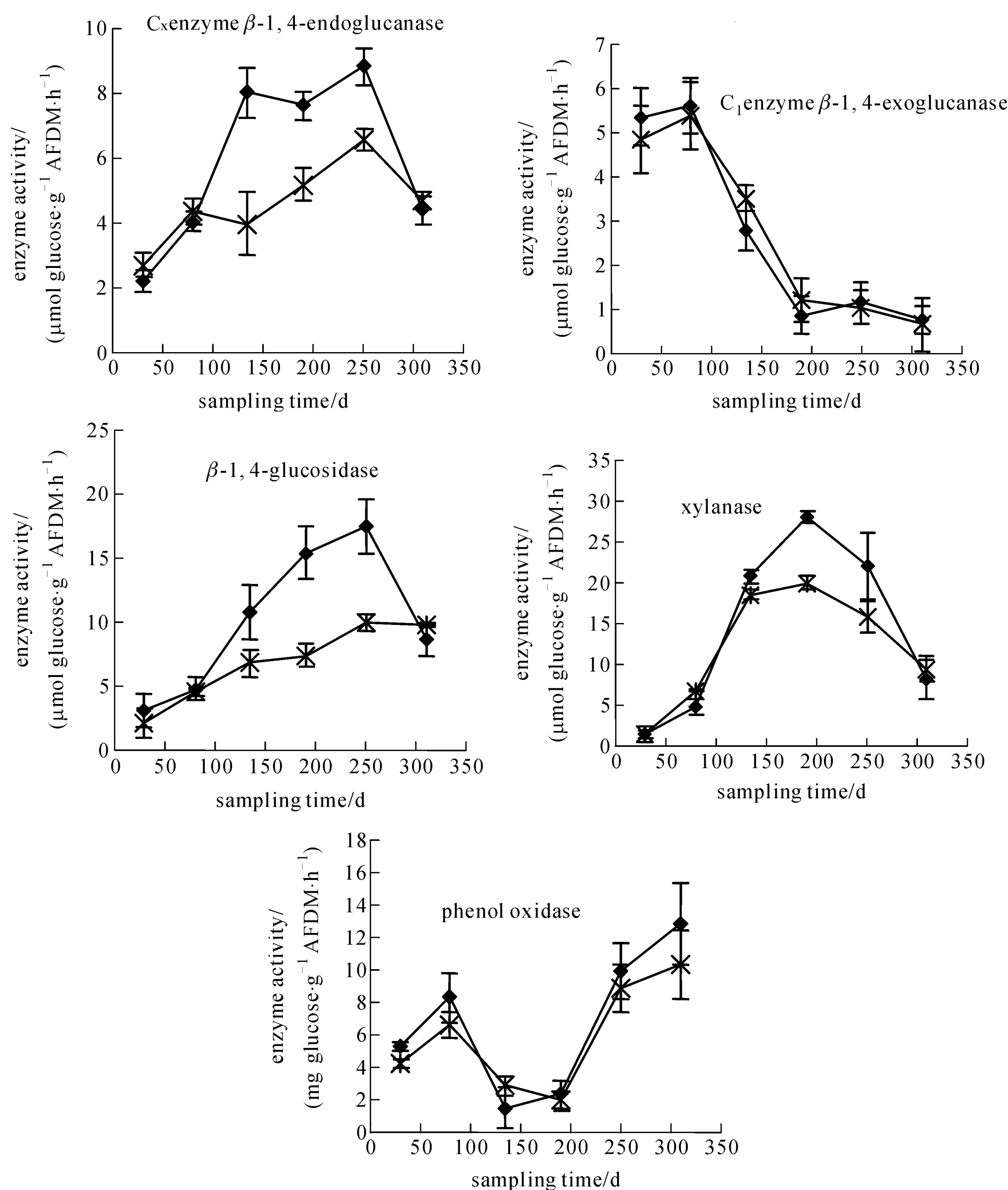

Fig. 5 Dynamics of β-1,4-endoglucanase, β-1,4-exoglucanase,β-1,4-glucosidase, xylanase and phenol oxidase activity during litter decomposition in field study. Bars are means with standard error ($n = 4$).

generally have a low P content (0.2% to 0.8%) and when microbes decompose and utilize this organic residue, they will synthesize phosphatase to obtain a chemical composition of P for self metabolism and to decompose organic residues further. Therefore phosphatase activity and microbial metabolism are related to be adaptable to their own regulation. In this experiment, it is likely that weak acidity (pH 4.75) of the soil matrix was the important environmental factor that led to the activity of acid phosphatase to be higher than that of alkaline phosphatase. That acid phosphatase activities in different treatments were higher in laboratory tests, compared to those in the field study, may be due to different conditions and at different stages of decomposition. Leaf litter decomposition was slow and the dry mass loss was only 25% during the entire experiment in the laboratory, while dry matter of leaf litter in the field study was ultimately reduced by 60% to 70% (Zhang et al., 2006). These results suggest that leaf litter in the laboratory study were still at the early stages of decomposition and the microbes were still synthesizing a large amount of induced phosphatase to decompose substrates further. At the middle and later stages of decomposition in the field study, acid phosphatase activity started to decline and it also began to weaken at the final stage of decomposition in laboratory experiments, when leaf litter decomposition entered the difficult decomposi-

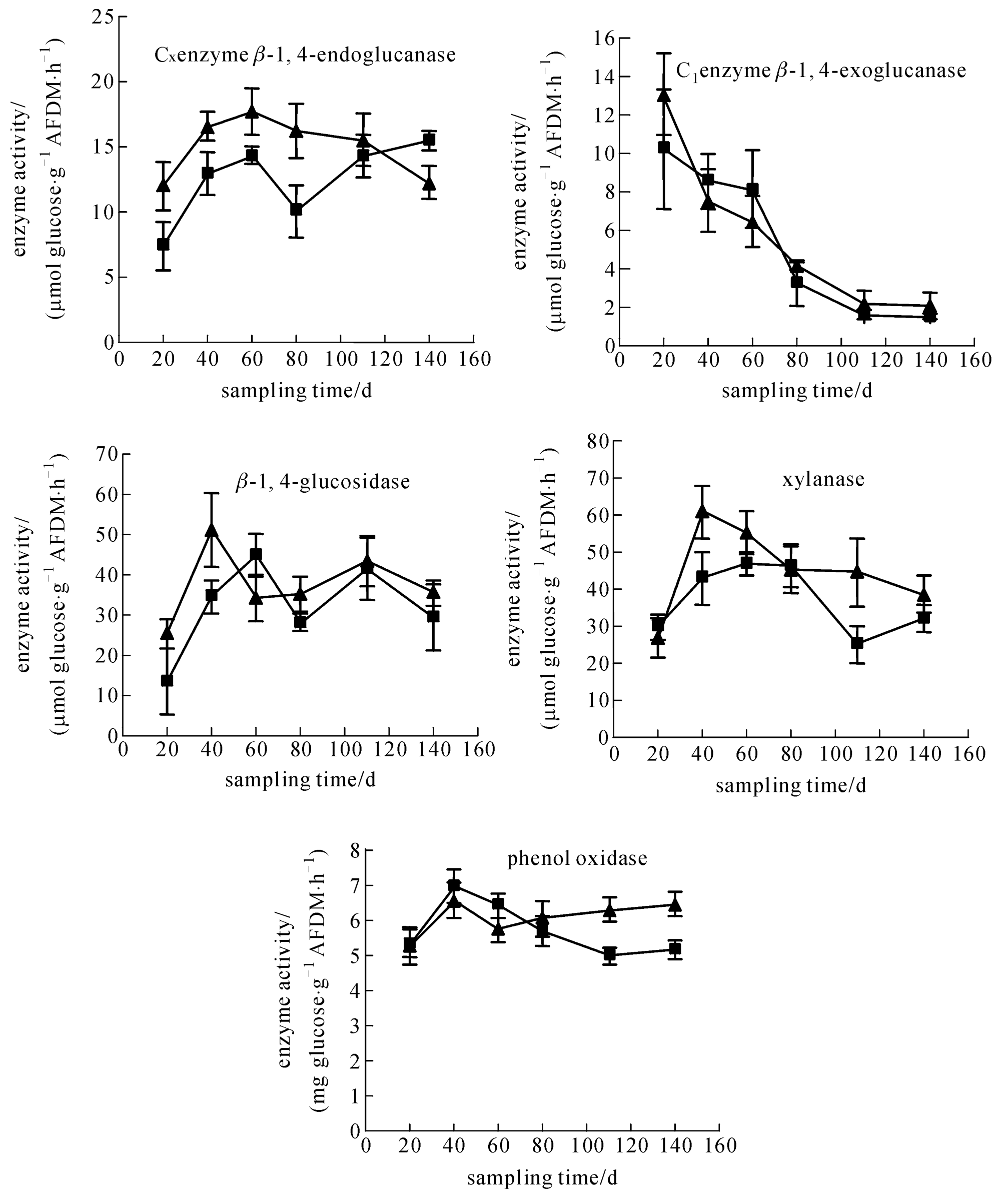

Fig. 6 Dynamics of β-1,4-endoglucanase, β-1,4-exoglucanase, β-1,4-glucosidase, xylanase and phenol oxidase activity during material decomposition in simulated study. Bars are means with standard error ($n = 3$).

tion stage (Zhang et al., 2006). Therefore, there must be a certain response between acid phosphatase activity and leaf litter decomposition process.

The main function of exoglucanase C_1 is hydrolyzing the non-decomposed natural cellulose macromolecules into amorphous cellulose. Thus, its activity is the highest in the initial stage of decomposition and declined gradually with the decomposition process.

Xylanase and polyphenol oxidase participate in the decomposition of recalcitrant compounds at the later stage of the decomposition. It was at the same decomposition stage of lignin, in the middle-term (135 d) of the leaf litter decomposition in the field tests that the xylanase activity increased rapidly until the later stage of decomposition (250–310 d). When the rate of decomposition clearly declined, it was, on the one hand, due to the more hardy decomposing material (polyphenols, tannins, etc.) and residues. On the other hand, it was affected by environmental conditions (rainfall decreased significantly in January to March in 2005) at this moment. As a result, xylanase activity declined rapidly at the final stage of decomposition, while polyphenol oxidase activity increased rapidly at this time. Xylanase and polyphenol oxidase activity in the F treatment (100 μm mesh) at the later stage in leaf litter decomposition was significantly lower than that in the C treatment (1 mm mesh). Therefore, the rate of decomposition of the F treatment decreased at the later stage, which ultimately affected the leaf litter

Table 4 Correlation coefficients (r) for enzyme activities and mass loss rates of decomposing material

enzyme type	treatment	mass loss/%
invertase	C	−0.331
	F	−0.713**
	MN	−0.493*
	M	−0.745**
amylase	C	−0.373
	F	−0.561*
	MN	−0.637**
	M	−0.059
acid phosphatase	C	−0.832**
	F	−0.660*
	MN	0.799**
	M	0.827**
alkaline phosphatase	C	−0.203
	F	0.096
	MN	0.902**
	M	0.246
exoglucanase	C	−0.934**
	F	−0.904**
	MN	−0.894**
	M	−0.946**
endoglucanase	C	0.587*
	F	0.667**
	MN	0.777**
	M	0.280
Β-glucosidase	C	0.746**
	F	0.912**
	MN	0.581*
	M	0.280
xylanase	C	0.573*
	F	0.643**
	MN	−0.198
	M	0.293
phenol oxidase	C	0.431
	F	0.335
	MN	0.580*
	M	−0.236

decomposition process (Zhang et al., 2006). Vaccinated nematode treatment (MN) endocellulase and xylanase activities were significantly higher than that of the unvaccinated nematode treatment (M) ($p < 0.05$) in laboratory tests, which was similar to the rapid decomposition process at the former stage and slowly at the later stage (Zhang et al., 2006).

The total amount of enzyme activity in the leaf litter indoor and outdoor differed in this study. We consider that it was mainly due to different environmental test conditions, since the decomposition of leaf litter in the field was affected by the drenching rain, perhaps causing the enzyme, as a protein, to be leached by rain and partly lost. The dynamics of enzyme activity indoor and outdoor may also vary, which was mainly because that leaf litter in the laboratory had not yet reached the decomposition level. Another reason may be that, compared with the experience in the field, leaf litter remains at the initial decomposition phase at the end of the simulation test in the laboratory and the actual process of decomposition tends to slow down or even stop (Zhang et al., 2006).

We also found from our analysis that shortening the food chain (100 μm mesh processing compared to 1 mm mesh processing; unvaccinated nematodes compared to vaccinated nematodes), significantly decreased the activity of endocellulase, *β*-glucosidase, xylanase and polyphenol oxidase and eventually slowed the decomposition process.

5.2 Relationship between enzyme activity dynamics and the decomposition process

The results of correlation tests (Table 4) show that, except for phosphatase which may be affected by environmental (soil) conditions and had no linear relationship with the decomposition process, invertase, amylase and exoglucanase had negative relationships and endocellulase, *β*-glucosidase, xylanase and polyphenol oxidase had positive relationships with litter decomposition (mass loss) (Zhang et al., 2006). These results suggest that invertase, amylase and exoglucanase played key roles for microbiota utilizing the substrates at the early stages of decomposition. Their functions gradually weakened during the decomposition process, while endocellulase, *β*-glucosidase, xylanase and polyphenol oxidase worked on the further decay of recalcitrant compounds at later stages.

These results show that it is not that a certain enzyme functions alone, but that various enzymes combine with micro-organisms to function in the decomposition of organic residues. Decomposition of organic residue is essentially a process of hydrolysis. Various types of enzymes, related to the carbon cycle, can be used as important indicators in the leaf litter decomposition process.

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