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Dynamics of Soil Microbial Biomass and Enzyme Activities at Different Developmental Stages of Tomato

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A pot experiment was conducted to study the dynamics of soil microbial biomass carbon, soil microbial biomass nitrogen, soil enzyme activities in the rhizospheric soil at different developmental stages of tomato under greenhouse conditions. It was evident that the tomato plants had significant rhizospheric effects on soil microbial properties. Soil microbial biomass carbon and nitrogen significantly decreased in the initial fruiting stage and early blossoming stage, respectively, following increased gradually and reach a maximum at the full bearing period, and then gradually decreased. During the whole growing season, rhizospheric soil invertase, urease and neutral phosphatase activities first increased and then decreased. At the late stage of tomato, soil microbial biomass and enzyme activities decreased. Significant correlations between soil microbial biomass and invertase, urease and neutral phosphatase activities, but less correlation was found between microbial biomass and hydrogen peroxidase activity.

Key words: Tomato, Soil microbial biomass, Soil enzyme activities.

The rhizosphere is an active interface of the soil–plant root–microorganism ecosystems for nutrient exchange. On one hand, plants affect the soil properties and microbial activity in the rhizosphere through respiration and the secretion of organic matter (Shi, 1993); on the other hand, soils provide nutrients to the plants through the rhizosphere in various ways (Zhang, 1998), and the transport of nutrients directly affect the growth and development of plants, as well as the survival and propagation of microorganisms. Therefore, studying rhizospheric soil conditions, especially changes in rhizospheric microorganisms, is of high significance for understanding the conversion of rhizospheric nutrients, availability of soil nutrients, and crop resistance to adversity.

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Soil microorganisms and soil enzymes are essential players in soil nutrient cycling and energy flow, promoting the mineralization and decomposition of soil organic matter and cycling and transformation of soil nutrients (Huang, 2000). The soil microbial biomass is the source and repository of plant nutrients, and soil microorganisms also actively participate in nutrient cycling, are extremely sensitive to the changes in the soil environmental factors and respond rapidly to even small changes (Dick *et al*. 1996). Soil enzymes are a class of biologically active substances derived from microorganisms, living plants and animals, or residues of plants and animals. They can catalyze all the biochemical reactions in soils and are important factors affecting the biological fertility of soils. In recent years, there have been studies on the impacts of different types of crops and farming management strategies on the eco-environment of rhizosphere in terms of soil biological characteristics (Vallejo *et al*., 2010; Normander *et al*., 2000; Marcial *et al*.,

2003). However, researches on the changes in rhizospheric soil conditions for protected tomato plants at different growth stages are rarely reported. Therefore, in this study, classical experimental techniques were used to conduct continuous dynamic tracking on the rhizospheric biological properties at different growth stages of tomato plants and to provide a theoretical basis for regulating the rhizospheric micro-environment and utilizing beneficial microorganisms to promote the growth of tomato plants.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse in the experimental field at Shenyang Agricultural University (41°312 N, 123°242 E), from March to August 2008. East-west trending cement tanks (length × width × height = 4 m × 0.4 m × 0.43 m) were built inside the green house and filled with brown soil for planting tomatoes. The soil with the initial basic properties as follows: soil organic carbon 26.55 $g \cdot kg^{-1}$, total nitrogen 1.43 $g \cdot kg^{-1}$, total phosphorus 0.81 $g \cdot kg^{-1}$, pH 6.8. The tomato seeds were sown on March 1, and the seedlings were transplanted into the cement tanks on April 17, with a plant spacing of 40 cm. There were 10 plants in each tank and a total of four tanks. One slot in each tank was randomly left unplanted as the blank control. The two rows of tomato plants on the east and west sides of the tank that were near the greenhouse vents were used as protection rows and were not sampled. Conventional cultivation and management were conducted and retained three spikes of fruit. Before transplanting, mixed soil samples were prepared as a control, using soils inside the tanks that were sampled with a five-point sampling process. Sampling was respectively conducted on tomato7-9 leaf stage, early blossoming stage, early fruiting stage, full bearing stage, and the last growth stage. Soils were randomly collected from the rhizosphere of three plants in each tank in triplicate. The detailed sampling method was applied as follows. First, cut the aerial parts of the tomato plants and dug out the complete tomato roots with a shovel, then gently removed chunks of soil between the roots and shook off the soils attached to the root surface onto the pre-prepared clean filter paper. Last carefully removed residual roots, sieved the fresh soil samples collected with a 1-mm sieve, and then stored them in a refrigerator at 4°C for later analysis of soil microbial biomass and enzyme activities.

Microbial biomass C and N were determined by the fumigation-extraction method (Brookes *et al*. 1985; Vance *et al*. 1987). Neutral phosphatase, invertase and urease activities were determined on 1g of soil and incubated with their buffered substrates (p-nitrophenyl phosphate, p-nitrophenyl-β-D- glucoside and urea respectively) at 37°C for 24h, the products were quantified colorimetrically. Soil catalase activity was measured by the method of titration with KMnO4. All enzyme activity assays had two controls: buffered substrate solution without soil and soil without buffer solution but no substrate.

Results of soil microbial biomass and enzyme activities were tested for the normal distribution prior to statistical analysis. All statistical analyses were performed by SPSS 11.0 for Windows (SPSS, 2001). Statistical significance of the difference was estimated by the least significant difference (LSD) test at the 5% or 1% level.

RESULTS

Rhizospheric soil enzyme activities at different growth stages of tomato

The soil invertase and neutral phosphatase activities showed the same trend of changes in different growth stages. Table 1 showed that, compared with the control, planting tomatoes significantly improved soil invertase and neutral phosphatase activities. Soil invertase and neutral phosphatase activities gradually increased with the growth and development of tomato. The differences in soil invertase activity were significant between different growth stages: the activity in the full bearing stage was a 101% increase compared with that before transplanting but declined rapidly at the late stage of tomato's growth. The magnitude of changes in the soil neutral phosphatase activity was smaller, and except for a significantly different enzyme activity between various growth stages and before transplanting, the differences among different growth stages were not significant.

Table 1. Soil enzyme activities at different growth stages of tomato

Sampling period	Invertase (Glucose,mg·g^{-1}·d^{-1})	Urease (NH_3-N,mg·g^{-1}·d^{-1})	Neutral phosphates (Phenol, mg·g^{-1}·d^{-1})	Hydrogen peroxidase ($KnMO_4$,ml·g^{-1}·h^{-1})
Before transplanting	8.01±0.05Ef	5.19±0.02Ee	0.29±0.007Dd	0.92±0.07De
7-9 leaf stage	11.72±0.19Dd	6.84±0.17Cc	0.32±0.003Cc	1.50±0.05BCbc
Early blossoming stage	13.48±0.33Cc	7.79±0.04Bb	0.34±0.001Bb	1.38±0.04Cc
Early fruiting stage	14.91±0.24Bb	8.34±0.14Aa	0.35±0.004Aa	1.90±0.05Aa
Full bearing stage	16.16±0.22Aa	7.82±0.12Bb	0.36±0.005Aa	1.62±0.06Bb
Last growth stage	9.18±0.25Ee	5.64±0.11Dd	0.34±0.003BCb	1.08±0.07Dd

Note: Uppercase and lowercase letters indicate significant at $p<0.05$ and $p<0.01$, respectively.

Table 1 showed that the soil urease and catalase activities at different growth stages of tomato were higher than before transplanting. Throughout the growing season, the urease activity first increased and then decreased: between leaf period to initial fruiting period, the urease activity increased gradually and reach a maximum in the initial fruiting stage, and then gradually decreased. The urease activities at early blossoming time were related to each other. The soil urease activity differences between other growth stages were highly significant. The order of soil catalase activity at different growth stages of tomato was early fruiting stage > full bearing stage> leaf stage > early flowering stage > final growing stage > before transplanting. The catalase activity at early fruiting period was 106% higher than that before transplanting. Difference in catalase activity at each stage was significant or very significant level.

Soil microbial biomass at different growth stages of tomato

Changes in soil microbial biomass at different growth stages of tomato are shown in Figure 1. Both the soil microbial biomass carbon

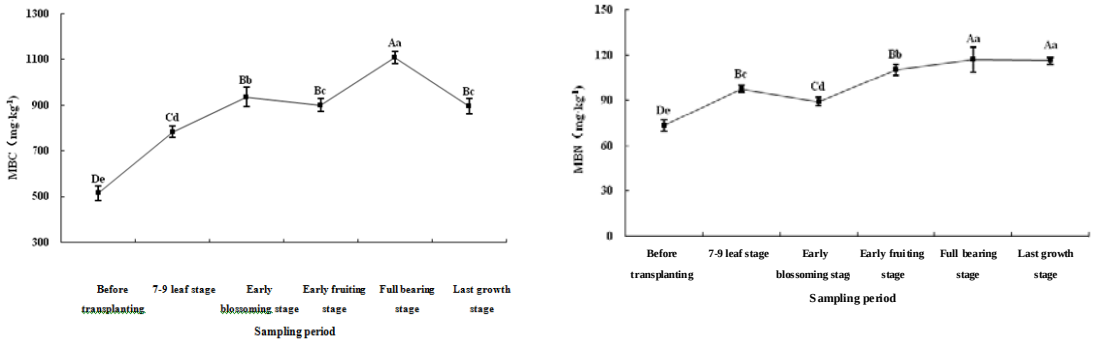

Fig. 1. The root cirumference soil microbial biomass of different growth stages of tomato. MBC on behalf of microbial biomass carbon and MBN on behalf of microbial biomass nitrogen. Uppercase and lowercase letters indicate significant at $p<0.05$ and $p<0.01$, respectively

and nitrogen were significantly higher than before transplanting. The soil microbial biomass carbon showed an "M-shaped" trend over the entire growth period: the biomass carbon gradually increased until early flowering stage and after a slight decline then increased significantly, at the high fruiting stage, biomass carbon reached the maximum and was 2.2 times its value before transplanting and than dropped to a level similar to that at early fruiting stage. Changes in microbial biomass nitrogen were slightly different from biomass carbon changes. In addition to lower at early blossoming stage, the biomass nitrogen was gradually increased in the whole growth period.

Correlation between soil enzyme activities and microbial biomass

Table 2 shows the correlation between soil enzyme activities and microbial biomass at different growth stages of tomato. Compared with biomass nitrogen, microbial biomass carbon had

stronger correlations with soil invertase, urease, and neutral phosphatase activities, which all reached the highly significantly level. The biomass nitrogen was highly significantly correlated with soil urease activity, with a correlation coefficient of 0.692, and significantly correlated with the activities of invertase and neutral phosphatase. Neither soil microbial biomass carbon nor nitrogen was correlated with catalase activity.

Table 2. The correlations between soil enzyme activities and microbial biomass

Microbial biomass	Invertase	Urease	Neutral phosphates	Hydrogen peroxidase
Biomass carbon	0.943**	0.927**	0.907**	0.390
Biomass nitrogen	0.57*	0.692**	0.531*	0.328

Note: ** and * indicate significant at P<0.01 and P<0.05 ,respectively

DISCUSSION

Soil enzymes are involved in all biochemical processes and directly affect the supply and storage of soil nutrients, therefore, soil enzymes are effective biological indicators of soil fertility. Changes in soil enzyme activity at different growth stages differ for different crops or the same crop in different environments (Jonasson *et al*., 1996; Dao, 2014). In this study, soil enzyme activities showed significantly different at different growth stages. The activities of soil invertase, urease, neutral phosphatase, and catalase showed an overall trend of first increasing and then decreasing with the development of tomato plant. The reason might be that although the tomato roots were still recovering and settling at the early post-transplanting stage and thus had less impact on soil enzyme activities, the large number of organic fertilizer that was applied before transplanting contained many active enzymes. Therefore, the soil enzyme activity at the early post-transplanting stage was still significantly higher than that before transplanting. Then, the roots secreted more enzymes as the tomato plants grew vigorously. The gradually increasing temperature inside the greenhouse and dressing promoted the growth and reproduction of microorganisms. All of these factors contributed to the growing soil enzyme activities. Entering the late stage of growth, the tomato plants' metabolism was slower, the rhizospheric activities slowed, and the nutrients from the fertilizers were exhausted. All these changes would lead to a significant reduction in soil enzyme activities. The decrease in catalase activity after initial flowering stage might have been related to changes in the root exudates during that time.

Soil microbial biomass is a repository of soil nutrients and an important source of available nutrients for plant growth. Compared with indicators of the number of microorganisms, soil microbial biomass can better reflect the actual number of microorganisms in soils and its potential role (Insam *et al*., 1991; He *et al*., 1997). Results showed that before tomato flowering, soil microbial biomass carbon gradually increased, because the base fertilizers provided a large number of available nutrients for the reproduction of soil microorganisms. After entering vigorous growth, tomato plants' demand for a variety of nutrients increased and the reaction against microorganisms weakened the plant's assimilation, while the increased temperature promoted the metabolic activities of soil microorganisms and enhanced respiration so that the plants consumed many of the available soil carbon sources (Liesack *et al*., 2000), resulting in a slight reduction in the soil biomass carbon at early fruiting stage. After fertilizer solves the problem of insufficient soil nutrients and alleviates the competition between tomato plants and soil microorganisms. The large amounts of root exudates and the residual effect of organic fertilizer provide a wealth of available carbon sources for the microorganisms (Baudoin *et al*., 2002), promoting the growth and reproduction of microorganisms. At the late stage of growth, the metabolism of tomato plants tended to slow. The significantly reduced root exudates reduced the carbon and energy sources available to microorganisms, resulting in decreased microbial biomass carbon. Changes in soil microbial biomass nitrogen at different growth stages of tomato were slightly different from the changes in biomass carbon. Compared with biomass carbon, the

biomass nitrogen content decreased significantly at initial blossoming stage. Nitrogen is the major nutrient required for the growth of tomato seedling. Consumption of large quantities of nitrogen causes the soil microbial nitrogen mineralization to be greater than nitrogen retention (Zhang *et al*., 2008), resulting in reduced microbial biomass nitrogen. However, the amounts of available soil nutrients, carbon sources, and energy substances available to microorganisms drastically increased with the dressing and progressing growth of tomatoes, and the microbial biomass nitrogen also gradually increased. Discrepancies exist between some reported results of soil microbial biomass at different crop growth stages and our results (Chen *et al*., 2010). These differences might be due to the differences in crops and growing environments. In this study, the correlation analysis of soil enzyme activities and microbial biomass showed that the activities of soil invertase, urease, and neutral phosphatase were correlated with biomass carbon and biomass nitrogen, and this result is consistent with the conclusion of Shen *et al*. (1999) in a maize field.

In summary, soil microbial biomass and enzyme activities in general exhibited a process of change that first increased and then declined with the growth and development progress of the tomato in the tomato–soil–microbial interaction system. When the tomatoes grew vigorously, the microbial biomass and enzyme activities were highest. It is evident that the tomato had significant rhizospheric effects on soil microorganisms. This characteristic of tomato can be used to guide the antagonistic rhizospheric bacteria screening to reduce the blindness in micro-ecological regulation and thus to reach the goal of improving rhizospheric microbial communities, implementing the ecological prevention, and developing tomato productivity.

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