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*Full Length Research Paper*

# Rhizosphere microbial dynamics of *Leymus chinensis* and its correlation with aboveground biomass and soil environment

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**The information on the rhizosphere microorganisms of *Leymus chinensis (L. chinensis)* was limited. To demonstrate the rhizosphere microbial dynamics and its relation to aboveground biomass and soil environment, the rhizosphere microbial numbers, biomass carbon, diversity index, aboveground biomass and soil temperature/moisture were measured and analyzed at different growth stages of *L. chinensis*. The results showed that the rhizosphere microbial numbers and biomass carbon were significantly related (P<0.05) among different growth stages based on one-way ANOVA analysis, showing single peak type in all growth stages with a maximum value at the fruiting stage. The rhizosphere effect was evident during active growing stages of *L. chinensis*. Regardless of other factors, aboveground biomass and soil temperature/moisture had significant effect (P<0.05) on the rhizosphere microbial numbers and biomass carbon based on correlation analysis. However, the results of stepwise regression equation suggested that the effect of aboveground biomass was dominated. The microbial community diversity index had no change (P>0.05) in all growth stages of *L. chinensis* based on one-way ANOVA and student's *t*-test, and no significant effect of aboveground biomass and soil temperature/moisture was found based on correlation analysis (P>0.05). To sum it up, the rhizosphere microbial numbers and biomass carbon showed significant dynamics with growth stages of *L. chinensis*, and the change were related primarily by plant growth. Nevertheless, the microbial community was indefinitely stable in all growth stages of *L. chinensis*.**

**Key words:** *Leymus chinensis*, rhizosphere microorganisms, aboveground biomass, soil temperature/moisture, dynamics, correlation.

## INTRODUCTION

The rhizosphere was defined by Hiltner in 1904 as the portion of soil influenced by the root, where micro-organisms interact with plant roots and soil constituents. Many rhizosphere microorganisms play an important role in key environmental processes, such as, the biogeochemical cycling of nutrients and matter, plant nutrition and health, and soil quality (Liu and Zhang, 2006). The populations and distribution of the rhizosphere microorganisms can be used as an important indicator in the quality of soil environment (Wen-Du et al., 2010) and plant nutrient uptake (Vega and Walter, 2007). Therefore, it is necessary to study the rhizosphere microorganisms on some kind of plants within certain regions.

Several studies have demonstrated that the rhizosphere microorganisms may show some seasonal dynamics (Gomes et al., 2003; Lipson and Schmidt, 2004; Picard et al., 2000; Marschner et al., 2001;

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Smit et al., 2001). Rhizosphere microorganisms have positive correlation with plant growth, and show significant rhizosphere effect. Environmental factors are believed to affect the rhizosphere microorganisms, especially soil temperature and moisture, which have significant effect on the numbers and activity of the rhizosphere microorganisms. Avrahami and Bohannan (2007) postulated that the influence of temperature is most dominant. On the contrary, some other researchers argued that soil temperature and moisture have no effect on the rhizosphere microorganisms (Santos-González and Roger, 2007; Zul et al., 2007). Thus, the factors affecting the rhizosphere microorganisms merit further investigation.

*Leymus chinensis* (Trin.) Tzvelev is a perennial grass widely distributed in the eastern region of the Eurasian Steppe Zone (Zhu, 2004). It is a dominant species from arid to semi-arid steppes in northern China with extensive plasticity in morphological and physiological characteristics. *L. chinensis* is a typical clonally plant, relying mainly on vegetative propagation for regeneration. *L. chinensis* regrows rapidly after being grazed or mowed early in the season tolerating drought, cold, and alkali stresses (Shi and Wang, 2005). To date, there is still lack of information on the existing of rhizosphere microorganisms of *L. chinensis*. Therefore, it is necessary to study the rhizosphere microbial dynamics and the general ecological relationships of the rhizosphere microorganisms with aboveground biomass of *L. chinensis* and soil environment in “healthy” grassland soil of *L. chinensis*.

## MATERIALS AND METHODS

### Study sites

Experiments were conducted in the Grassland Ecological Research Station of Northeast Normal University, Changling Country, Jilin Province, PR China (44°40′ to 44°44′ N, 123°44′ to 123°47′ E). The main vegetation type is meadow steppe dominated by *L. chinensis.* The study area has a semi-arid continental climate. The soil was salinized with a pH value of 7.5~9.0.

### Sampling

Samples were taken on May 7 (Stage I: Reviving stage), June 7 (Stage II: Heading stage), June 22 (Stage III: flowering stage), July 7 (Stage IV: Milking stage), July 22 (Stage V: Ripening stage), August 30 (Stage VI: Fruiting stage) and October 7 (Stage VII: Wilting stage) in 2006. In defined 10m×10m plots, we selected 1m×1m plot by five point sampling at each growth stage, after which we proceeded to take aboveground biomass and the rhizosphere soil samples. All aboveground vegetation within the plot was cut, and placed in plastic bags. The rhizosphere soil samples were colletecd using a core at the base of the plant to a 10 cm depth, where most of the roots were found. Given the dense root system of *L. Chinensis*, core soil after sieving (<4 mm) and manually removing roots and other plant particles was considered as the rhizosphere soil. The other core soil (>4 mm) are non-rhizosphere soils and used as a control group. Soil samples were kept at 4°C.

### Experiment

Aboveground biomass of *L. Chinensis* was determined by weighing dried (65°C for 24 h) and labeled in terms of g $m^{-2}$. Soil temperature was measured using a soil temperature thermocouple probe to a depth of 20 cm below the soil surface. Soil moisture content was determined by weighing fresh and dried soil (100°C for 24 h) and labeled in terms of % $g^{-1}$.

Colony forming units (CFU) were counted after serial dilution (10-fold) of 1 g dry soil suspension and plating on beef-protein agar medium for bacteria, Gause's synthetic agar medium for actinomycete, Martin agar medium for fungi, denitrifying bacteria medium for denitrifying bacterium, Hutchinson medium for aerobic cellulose-degrading bacteria, nitrite medium for nitrifying bacteria, Ashby medium for nitrogen-fixing bacteria and peptone medium for ammonifying bacteria. Soil microbial community diversity index of Shannon-Wiener Index, Simpson diversity index, and Pielou evenness index was calculated using the numbers of denitrifying bacterium, aerobic cellulose-degrading bacteria, nitrifying bacteria, Nitrogen-fixing bacteria and ammonifying bacteria in accordance with the following formula (Yao and Huang, 2006).

Shannon-Wiener index: $H=-\Sigma Pi \times \ln Pi$ (Pi: Proportion of the numbers in “i” species and total species)

Simpson diversity index: $D=1-\Sigma Pi^2$

Pielou evenness index: $E=H / \ln S$ (S: Total species)

Soil microbial biomass carbon was determined by the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976).

### Statistical analysis

All data were analyzed by SPSS (Version 13.0, Chicago, IL). One-way ANOVA analysis was carried out to calculate any differences among different growth stages. Student’s *t*-test was carried out to calculate the differences between the rhizosphere soil and the non-rhizosphere soils during different growth stages. Correlation analysis and stepwise regression equation was carried out to evaluate the correlation between the rhizosphere microorganism and aboveground biomass, soil temperature and moisture.

## RESULTS

### Dynamics in the total numbers of bacteria, actinomycetes and fungi during growth stages of *L. chinensis*

As indicated in Figure 1, the total numbers of the rhizosphere bacteria, actinomycetes and fungi, increased gradually along with the growth of *L. chinensis* and reached a maximum peak at the fruiting stage, with the total numbers of $10.20\times10^8$, $12.00\times10^6$ and $7.6\times10^5$ CFU $g^{-1}$ dry soil, respectively. After that, the total numbers were reduced to $0.40\times10^8$, $3.53\times10^6$ and $2.5\times10^5$ CFU $g^{-1}$ dry soil at the Wilting stage, respectively. The total numbers of the rhizosphere bacteria, actinomycetes and fungi were significantly different ($P<0.05$) among different growth stages based on one-way ANOVA analysis. The

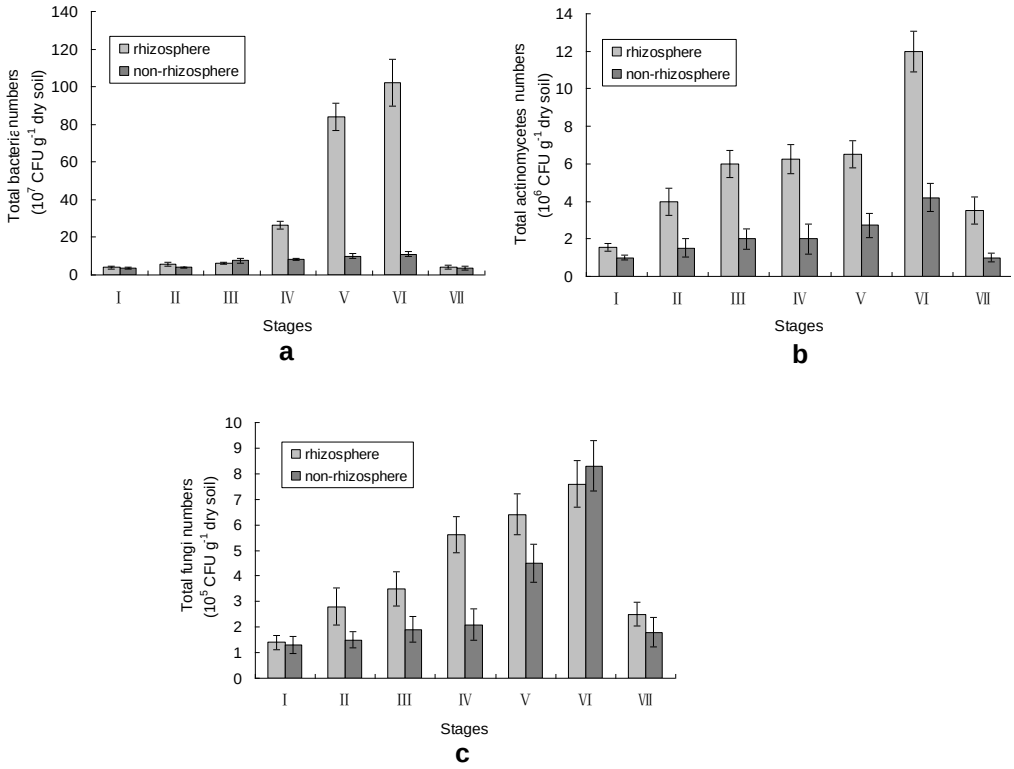


**Figure 1.** Dynamics in the total numbers of bacteria, actinomycetes and fungi of rhizosphere and non-rhizosphere soils during growth stages of *L. Chinensis.* I, Reviving stage; II, heading stage; III flowering stage; IV, milking stage; V, ripening stage; VI, fruiting stage; VII, wilting stage.
*Significantly relevant at P<0.05 confidence was calculated using student's *t*-test.

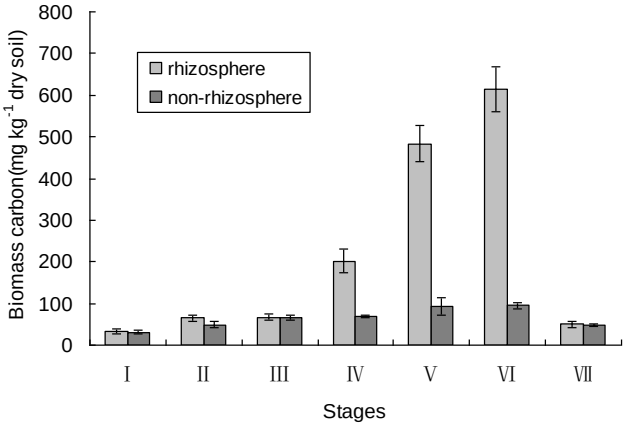


**Figure 2.** Dynamics in the microbial biomass carbon of rhizosphere and non-rhizosphere soils during growth stages of *L. Chinensis.* I, Reviving stage; II, heading stage; III, flowering stage; VI, milking stage; V, ripening stage; VI, fruiting stage; VII, Wilting stage.
*Significantly relevant at P<0.05 confidence was calculated using student's *t*-test.

total numbers of bacteria were significantly higher than that of actinomycetes and fungi in all growth stages.

As indicated in Figure 1, the total numbers in rhizosphere soil were greater than that in non-rhizosphere soils during most growth stages. Between rhizosphere and non-rhizosphere soils, the differences of bacteria numbers were significant (P<0.05) by student's *t*-test in milking stage, ripening stage, and fruiting stage. The differences in actinomycetes numbers were significant (P<0.05) by student's *t*-test in all growth stages. The differences of fungi numbers were significantly (P<0.05) by student's *t*-test in heading stage, flowering stage, milking stage and ripening stage. The biggest difference achieved was 9.27 times (Bacteria, Fruiting stage). The rhizosphere effect was evident. However, the total bacteria numbers in rhizosphere soils were lower than that in non-rhizosphere soils at the Flowering stage, and the total fungi numbers in rhizosphere soils were lower than that in non-rhizosphere soils at the fruiting stage.

## Dynamics in soil microbial biomass carbon during growth stages of *L. chinensis*.

As indicated in Figure 2, dynamics in rhizosphere soil microbial biomass carbon was consistent with

**Table 1.** Dynamics in Shannon-Wiener index, Simpson's diversity index and Pielou evenness indices of rhizosphere and non-rhizosphere soils during growth stages of *L. chinensis*.

| Stage | Shannon-Wiener index | | Simpson diversity Index | | Pielou evenness indices | |
|---|---|---|---|---|---|---|
| | Rhizosphere | Non-rhizosphere | Rhizosphere | Non-rhizosphere | Rhizosphere | Non-rhizosphere |
| Reviving | 0.4314 | 0.4855 | 0.1942 | 0.2275 | 0.2680 | 0.3016 |
| Heading | 0.4293 | 0.4991 | 0.2001 | 0.2294 | 0.2668 | 0.3101 |
| flowering | 0.4830 | 0.5476 | 0.2334 | 0.2592 | 0.3001 | 0.3402 |
| Milking | 0.4815 | 0.5168 | 0.2257 | 0.2388 | 0.2992 | 0.3211 |
| Ripening | 0.4949 | 0.5363 | 0.2270 | 0.2468 | 0.3075 | 0.3332 |
| Fruiting | 0.7566 | 0.7344 | 0.4353 | 0.3996 | 0.4701 | 0.4563 |
| Wilting | 0.6132 | 0.4450 | 0.2724 | 0.1979 | 0.3810 | 0.2765 |

**Table 2.** Dynamics in aboveground biomass, soil temperature and soil moisture during growth stages of *L. chinensis*.

| Stage | Aboveground biomass ($g/m^2$) | Soil temperature (℃) | Soil moisture (%) |
|---|---|---|---|
| Reviving | 65.97 | 13.61 | 10.46 |
| Heading | 71.90 | 16.45 | 13.92 |
| flowering | 74.82 | 20.36 | 15.63 |
| Milking | 77.91 | 21.57 | 17.45 |
| Ripening | 82.80 | 22.53 | 18.25 |
| Fruiting | 90.30 | 26.36 | 24.62 |
| Wilting | 68.50 | 13.28 | 5.52 |

rhizosphere microbial total numbers. In both the rhizosphere and non-rhizosphere soils, soil microbial biomass carbon reached a maximum value at the fruiting stage, by increasing to 614.6 and 94.4 mg $kg^{-1}$ dry soil. After that, soil microbial biomass carbon reduced to 49.8 and 47.6 mg $kg^{-1}$ dry soil, respectively. The soil microbial biomass carbon were significantly different ($P<0.05$) among different growth stages based on one-way ANOVA analysis. The differences were significantly ($P<0.05$) by student's *t*-test in milking stage, ripening stage, and fruiting stage. The biggest difference achieved was 6.51 times at the fruiting stage.

## Dynamics in soil microbial community diversity during growth stages of *L. chinensis*

Three diversity indices of Shannon-Wiener index, Simpson diversity index, and Pielou evenness Index were used to compare the differences of soil microbial community diversity between the different growth stages of *L. chinensis*. As indicated in Table 1, the changes of three diversity indices was relatively smaller between different growth stages, and a less change between rhizosphere and non-rhizosphere soils was also found [the changes were not significantly ($P>0.05$) based on one-way ANOVA analysis and student's *t*-test].

## The correlation between the rhizosphere microorganism and aboveground biomass, soil temperature and moisture

The results of aboveground biomass, soil temperature and moisture are shown in Table 2. In order to elucidate the associations between the rhizosphere microorganism and aboveground biomass, soil temperature and moisture, the measured data were calculated by correlation analysis. A number of linear regression equations were established based on analysis results (Table 3). The result showed that the rhizosphere microbial numbers and biomass carbon had significant positive correlation ($P<0.01$) with aboveground biomass of *L. chinensis*. The rhizosphere bacteria numbers and biomass carbon had significant positive correlation ($P<0.05$) with soil temperature and moisture. The total numbers of rhizosphere actinomycetes or fungi and biomass carbon had significant positive correlation ($P<0.01$) with soil temperature and moisture. However, the microbial community diversity indices had no correlation ($P>0.05$) with aboveground biomass, soil temperature and moisture.

In order to further elucidate how aboveground biomass, soil temperature and moisture affect the rhizosphere microbial numbers and biomass carbon, the measured data was calculated by stepwise regression analysis with a significant P value set at $P<0.05$. The results (Table 4)

**Table 3.** Correlation analysis between the rhizosphere microorganisms (total numbers of bacteria, actinomycetes and fungi, biomass carbon, diversity indices) and aboveground biomass, soil temperature and moisture. Significant correlation was set at P<0.05 and P<0.01.

| Microbial indicator | Influencing factors | Regression equation | P value |
|---|---|---|---|
| Total bacteria numbers | Aboveground biomass | y = 45.944x – 3161.1 | P<0.01 |
| | Soil temperature | y = 71.704x - 1042.3 | P<0.05 |
| | Soil moisture | y = 55.761x - 511.19 | P<0.05 |
| Total actinomycetes numbers | Aboveground biomass | y = 0.3753x – 22.844 | P<0.01 |
| | Soil temperature | y = 0.63x - 6.3821 | P<0.01 |
| | Soil moisture | y = 0.4798x - 1.562 | P<0.01 |
| Total fungi numbers | Aboveground biomass | y = 0.0265x - 1.5863 | P<0.01 |
| | Soil temperature | y = 0.0445x - 0.4278 | P<0.01 |
| | Soil moisture | y = 0.0331x - 0.0746 | P<0.01 |
| Biomass carbon | Aboveground biomass | y = 2.6459x - 179.5 | P<0.01 |
| | Soil temperature | y = 4.1616x - 58.099 | P<0.05 |
| | Soil moisture | y = 3.2311x - 27.198 | P<0.05 |
| Diversity indices | Aboveground biomass | | P>0.05 |
| | Soil temperature | | P>0.05 |
| | Soil moisture | | P>0.05 |

showed that two variables of soil moisture and temperature were excluded, and only the aboveground biomass was used as a variable in stepwise regression equation. The coefficients of stepwise regression equation were as same as linear regression equation. The significance tests showed that the regression equations reached a significant level (P<0.05).

## DISCUSSION

Dynamics of the rhizosphere microbial total numbers and biomass carbon in *L. chinensis* were shown to be a single peak type in all growth stages, and reach a maximum value at the fruiting stage. The total numbers and biomass carbon were significantly different (P<0.05) among the different growth stages of *L. chinensis*, showing obvious dynamics reported by several studies (Gomes et al., 2003; Lipson and Schmidt, 2004; Picard et al., 2000; Marschner et al., 2001; Smit et al., 2001). The total numbers and biomass carbon in rhizosphere soils were above average compared to that in non-rhizosphere soils, and were significantly different (P<0.05) during active growing stages of *L. chinensis*, the rhizosphere effect was evident. These results suggested that the rhizosphere microorganisms were closely related to physiological activity of *L. chinensis*, which can be associated with the amount and type of root exudates (Vega and Walter, 2007). The results of correlation analysis between the rhizosphere microorganism and aboveground biomass (P<0.01) also supported this opinion. On the contrary, the total bacteria numbers in rhizosphere soils were less than that in non-rhizosphere soils at flowering stage, and the total fungi numbers in rhizosphere soils also were less than that in non-rhizosphere soils at the fruiting stage, both showing the opposite effect. Hartwig et al. (1990) hypothesized that while root exudates could efficiently promoted the growth and reproduction of microorganisms, it might depress these numbers. Zhang et al. (2002) also argued that environmental factor and external interference could affect rhizosphere environment, leading to the opposite effect.

Several studies suggested that soil microbial diversity had seasonal fluctuations (Lipson and Schmidt, 2004; Picard et al., 2000; Marschner et al., 2001; Smit et al., 2001). However, our findings showed that three diversity indices of Shannon-Wiener index, Simpson diversity index, and Pielou evenness index had no significant changes (P>0.05) between rhizosphere and non-rhizosphere soils during growth stages of *L. chinensis*. The results of correlation analysis showed that the microbial community diversity indices had no correlation (P>0.05) with aboveground biomass, soil temperature and moisture. The results supported the opinion by Zul et al. (2007), and showed that soil microbial community was indefinitely stable in all growth stages of *L. chinensis,* and no evident effect of aboveground biomass, soil temperature and moisture was found.

The rhizosphere microbial total numbers and biomass

**Table 4.** Stepwise regression equation between the rhizospheric microbial numbers, biomass carbon and aboveground biomass, soil temperature and moisture.

| Stepwise regression equation | | Rhizospheric Bacteria $Y_1$ | Rhizospheric actinomycetes $Y_2$ | Rhizospheric Fungi $Y_3$ | Biomass carbon $Y_4$ |
|---|---|---|---|---|---|
| Variables entered | | $X_1$ | $X_1$ | $X_1$ | $X_1$ |
| excluded Variables | | $X_2$ $X_3$ | $X_2$ $X_3$ | $X_2$ $X_3$ | $X_2$ $X_3$ |
| R value | | 0.927[a] | 0.961[a] | 0.977[a] | 0.977[a] |
| Coefficients | Constant | -3161.054 | -22.844 | -1.586 | -179.501 |
| | $X_1$ | 45.944 | 0.375 | 0.026 | 2.646 |
| Sig. | | 0.003[a] | 0.001[a] | 0.000[a] | 0.001[a] |

$X_1$, Aboveground biomass; $X_2$, soil moisture; $X_3$, indicates temperature.

carbon had a significant positive correlation ($P<0.05$) with soil temperature and moisture according to correlation analysis. It showed that soil temperature and moisture had significant effect on the rhizosphere microbial total numbers and biomass carbon regardless of other factors. The results of Chen et al. (1995) also supported this point. Nevertheless, the results of stepwise regression equation suggested that when soil temperature and moisture were compared, the effect of aboveground biomass dominated the rhizosphere microbial total numbers and biomass carbon. One reason might be that root exudates could efficiently promote the growth and reproduction of microorganisms (Sood, 2003; Gu and Mazzola, 2003), and microbial activity could also affect plant absorbing nutrients (Ström et al., 2001), leading to the restrictive relation between plant and the rhizosphere microbial total numbers and biomass carbon. Paul and Clark (1996) postulated that plant could affect rhizosphere microorganisms by changing soil nutrients, soil temperature and moisture, etc. On the other hand, the changes of soil temperature (13~26℃) and moisture (5~25%) were probably limited on narrow range, so their influence was weakened compared to aboveground biomass.

The results of our study showed that the rhizosphere microbial numbers and biomass carbon had significant dynamics with growth stages of *L. chinensis*, and the rhizosphere effect was evident during active growing stages. While aboveground biomass, soil temperature and moisture had significant effect on the rhizosphere microbial numbers and biomass carbon, however, the effect of aboveground biomass was dominated. The microbial community was indefinitely stable in all growth stages *L. chinensis,* and no evident effect of aboveground biomass, soil temperature and moisture was found.

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