Comparison of soil microbial biomass C, N and P between natural secondary forests and *Larix olgensis* plantations under temperate climate

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Abstract
Conversion of natural secondary forests (NSF) to mono-cultural *Larix olgensis* plantations (LOP) is a common forest management driven by an increasing demand for timber. To assess the impact of conversion of NSFs to LOPs on soil microbial properties under temperate climate, the microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) were compared between NSF stands and LOP stands in Northeast China. The results indicated that the MBC, MBN and MBP were significantly lower in LOP stands than in NSF stands for both 0-15 cm and 15-30 cm soil layers. The percentage ratios of MBC, MBN, and MBP to soil organic C, total N or total P were significantly reduced in LOP stands; and they varied with time during the growing season significantly. The increase of microbial biomass in summer may be an important retention mechanism for conserving soil nutrients in the studied area. The above mentioned results suggest that NSF stands are better in conserving soil microbial biomass and nutrient than those of LOP stands.

Key Words
Microbial biomass carbon, nitrogen, P, Natural secondary forest, Larch plantation forest.

Introduction
Soil microbial biomass, both a source and sink of available nutrients for plants, plays a critical role in nutrient transformation in terrestrial ecosystems (Singh *et al.* 1989). Any changes in the microbial biomass may affect the cycling of soil organic matter. Thus, the soil microbial activity has a direct influence on ecosystem stability and fertility (Smith *et al.* 1993). Generally, microbial biomass can offer a means in assessing the soil quality in different vegetation types (Groffman *et al.* 2001).
Mixed broadleaved-Korean pine forest is one of the most important regional climax forest types in Northeast China, of which more than 70% have become natural secondary forest (NSF) after a century of timber exploitation (Zhu *et al.* 2007). To meet the growing demands for timber, many of the NSFs have been replaced by larch plantations (LOP) with fast-growing and high-yield reputation. Given the extensive coverage and economic value, the LOPs have in recent years attracted much attention in their role for contributing to ecosystem service as well as timber production (Zhu *et al.* 2007). However, decline in yield and soil fertility occurs in the LOPs. Generally, soil microbial biomass has been considered as the major indicator in the evaluation of soil restoration (Ross *et al.* 1982). Some researchers have found that soil microbial biomass decreased in the plantations in comparison with the natural forests in tropical and subtropical forest ecosystems (Behera and Sahani 2003). However, little information is available about the impacts NSFs and LOPs on soil microbial properties in the temperate forest ecosystems. In order to understand the mechanism of the yield and soil fertility decline in LOPs, and to maintain the long-term productivity of these forest soils, the impacts of forest conversion from NSF to LOP on soil microbial biomass under temperate climate were tested because the microbiological indicators have been applied by researchers in number studies of soil restoration in forest ecosystems (Wang and Wang 2007).

Methods
Site description and experimental design
The study was conducted at the Qingyuan Experimental Station of Forest Ecology, Institute of Applied Ecology, Chinese Academy of Sciences. The station is located in a mountainous region in the eastern Liaoning Province, Northeast China (41º51' N, 124º54' E, 500-1100 m a.s.l.). It is a continental monsoon type with a humid and rainy summer, and a cold and dry winter. The monthly mean temperature and precipitation in the growing season of 2008 are shown in **Table 1**. The brown forest soil belongs to Udalfs according to U.S. Soil Taxonomy.

The study site was originally occupied by primary mixed broadleaved-Korean pine forests until 1930s and subsequently subjected to decades of unregulated timber removal. A large fire that occurred in the early 1950s completely cleared off the original forests and the site was replaced by a mixture of naturally regenerating broadleaved native tree species (secondary forests). Since 1960s, patches of the NSFs were cleared for larch plantations. The sample plots were set up on three NSF stands and three LOP stands with ages 16- 44 years. The six stands have the same topographical features and are on soils developed from the same parental materials. In each of the stands, three 20 × 20 m plots were laid out in September 2006. The NSF plots consisted of *Juglans mandshurica*, *Quercus mongolica*, *Acer mono* and *Fraxinus rhynchophylla* in the tree layer, *A. triflorun*, *A. tegmentosum* and *Syringa amurensis* in the understory component. The LOP plots contain *Acer tegmentosum*, *A. pseudo-sieboldianum* and *Schisandra chinensis* in the shrub layer.

Table 1. Monthly mean air temperature and precipitation in the study area in 2008

Month	Apr	May	Jun	Jul	Aug	Sep	Oct
Temperature (Celsius degree)	9.90	13.04	16.38	20.27	18.07	16.09	11.55
Precipitation (mm)	58.6	161.3	158.3	264.4	185.1	31.8	19.2

Soil sampling and analyses

Soil samples were collected from the plots in April, July and September 2008, representing spring, summer and autumn seasons respectively. Litter was removed before sampling. Nine soil samples were randomly collected from 0 to 15 cm and 15-30 cm depths at each plot and mixed thoroughly to obtain a homogeneous sample for each soil depth. The mixed soil sample was divided into two parts. One was sieved through a 2-mm mesh immediately and stored at 4 ℃ until analysis for the estimation of microbial biomass C (MBC), microbial biomass N (MBN) and microbial biomass P (MBP). The other was air-dried and passed through a 0.25 mm sieve for the analyses of soil organic C (SOC), total N (TN) and total P (TP). SOC and TN were analyzed by dry combustion on a Vario EL elemental analyzer (Germany). The TP was determined following H_2SO_4-$HClO_4$ digestion (Olsen and Sommers 1982). MBC, MBN and MBP were determined by fumigation-extraction method (Vance *et al.* 1987).

Statistical analyses

All observed data are expressed on air-dry soil weight basis. Statistical analyses were performed by using the SPSS 11.5 for Windows. One-way ANOVA was performed by soil layers, and LSD's (least significant difference) test was applied post hoc to distinguish the differences of soil chemical properties between NSF and LOP. The three-way analyses of variance (three-way ANOVA) was used to compare the general effects of forest types, sampling seasons and soil layers on microbial biomass C, N and P.

Results

Soil chemical properties

SOC concentration was higher in the NSF than in the LOP for 0-15 cm soil layer (P<0.05), while concentrations of TN and TP did not significantly vary between the NSF and LOP stands for both 0-15 cm and 15-30 cm soil layers. The C/N, C/P and N/P ratios were significantly higher in NSF stands than those in LOP stands (P<0.05), and decreased with soil depth for both NSF and LOP stands (**Table 2**).

Table 2. Soil chemical properties in the natural secondary forest (NSF) and the *Larix olgensis* plantation (LOP)

Soil depth (cm)	Forest type	SOC (g/kg)	TN (g/kg)	TP (mg/kg)	C/N	C/P	N/P
0-15	NSF	50.5±6.2*	4.21±0.60	743±108	12.3±0.3*	70.2±3.2*	5.70±0.19*
	LOP	34.7±2.3	3.20±0.21	1010±85	10.9±0.2	35.2±1.8	3.23±0.14
15-30	NSF	23.4±3.8	2.18±0.40	645±113	11.1±0.3*	37.2±1.8*	3.35±0.13*
	LOP	24.0±2.4	2.38±0.22	918±73	10.0±0.2	25.9±1.1	2.59±0.09

SOC: soil organic C; TN: total N; TP: total P. Values shown in tables are means ± standard errors (n=9).
* Indicate significant differences between NSF stands and LOP stands at the corresponding depth at P=0.05 levels.

Soil microbial biomass

The concentrations of MBC, MBN and MBP were significantly greater in NSF stands than in LOP stands (**Figure 1**). There was significant seasonal variation in soil MBC for both the forest types (**Figure 1A**, P<0.05). However, there was no clear seasonal variations for MBN and MBP in both NSF and LOP stands (**Figure 1B, C**). Soil MBC concentration was significantly higher in summer than that in spring and autumn in the two soil layers for both the forest types (**Figure 1A**); whilst the MBN and MBP concentrations were not significantly different among the three seasons (**Figure 1B, C**).

The ratios of MBC/SOC, MBN/TN and MBP/TP were consistently higher in soils of the NSF stands than those of LOP in different seasons, with the exception of MBC/SOC, which was lower in spring in NSF

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stands (**Figure 2**). These ratios showed significant variations in sampling seasons both in NSF and LOP stands, which were higher in summer than those in spring and autumn (**Figure 2**).

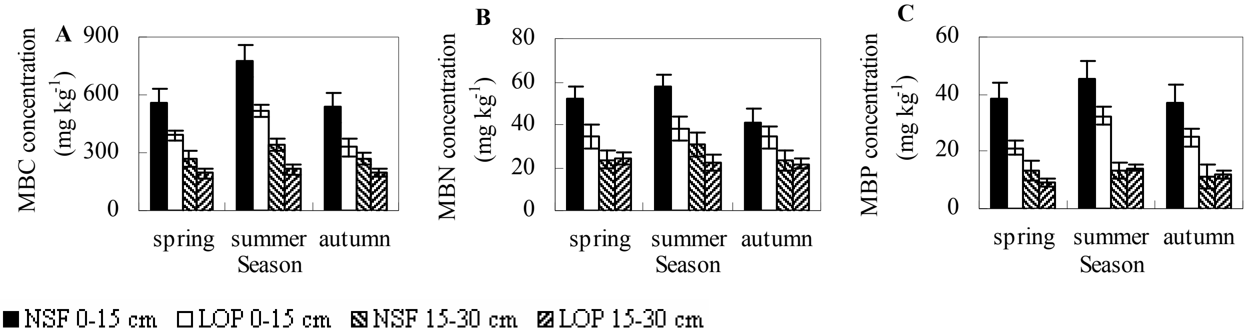

Figure 1. Microbial biomass C, N and P in soils of natural secondary forests (NSF) and *Larix olgensis* plantations (LOP) at different seasons. A: MBC, B: MBN, C: MBP (Vertical bars indicated the standard errors)

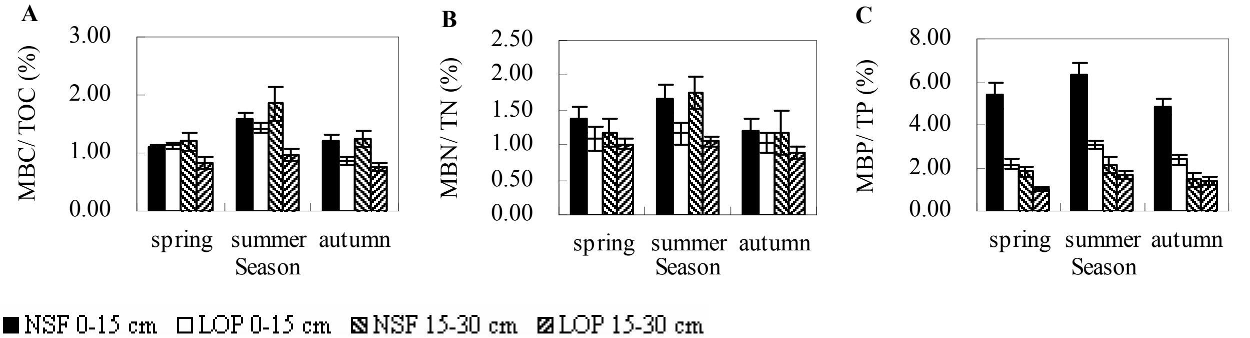

Figure 2. The ratios of MBC/SOC (A), MBN/TN (B) and MBP/TP (C) in the soils of natural secondary forest (NSF) and *Larix olgensis* plantation (LOP) at different seasons (Vertical bars indicated the standard errors)

Results from three-way ANOVA demonstrated that both forest types and sampling seasons had significant effects on MBC concentrations and the ratios of MBC/SOC, MBN/TN and MBP/TP. The soil MBN and MBP were significantly affected by forest types as well; but they were not significantly influenced by the sampling seasons (**Table 3**). The concentrations of MBC, MBN and MBP were all varied with the soil depth. There was a significant interaction between forest type and soil depth for MBC, MBN and MBP (**Table 3**).

Table 3. Results of three-factors ANOVA (soil depth, forest type and season) for microbial biomass and the ratio of microbial biomass to soil nutrients (P<0.05, ***P<0.01)**

Factors (F-ratios)	MBC	MBN	MBP	MBC/SOC	MBN/TN	MBP/TP
Forest type	30.19**	10.38**	10.83**	25.83**	10.63**	75.64**
Sampling Season	8.50**	2.21	2.66	14.77**	3.23*	6.83**
Depth	95.74**	45.27**	85.94**	1.25	0.58	157.08**
Forest × Season	0.65	1.01	0.51	2.12	1.22	1.39
Forest × Depth	4.62*	4.24*	8.80**	8.83**	0.11	41.23**
Season × Depth	3.23*	0.45	0.84	0.13	0.09	1.02
Forest × Season × Depth	0.05	0.38	0.01	1.30	0.17	0.11

Discussion and conclusions

Given the major differences in composition of tree species, we expected the differences in soil microbial biomass for the two forest types. The results that MBC, MBN, MBP, MBC/SOC, MBN/TN and MBP/TP ratios were significantly higher in NSF stands than those in LOP stands suggest the advantage of NSF in conserving soil fertility. Generally, soil microbial biomass depends on soil organic matter as substrate; therefore, the decrease of SOC will cause the reduction of soil microbial biomass (Chen *et al.* 2005). Thus, the main factor that induced the higher concentrations of MBC, MBN and MBP in NSF stands seems to be the greater availability of organic matter in NSF stands. The reasons for the decline of SOC in LOP stands may be explained as follows: firstly, poor site preparation practices such as removal of litter and ploughing before planting, which resulted in rapid and large loss of soil organic matter. Secondly, NSF stands contain more tree species than those in LOP stands; and the number of tree species affect the availability and biochemical composition of organic matter inputs into soil (Leckie *et al.* 2004). Thirdly, the decomposition of litter in LOP stands was relatively slower in comparison with NSFs (Liu *et al.* 1998).

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The MBC/SOC ratio or microbial quotient has been widely used as an indicator of the changes in organic matter status due to alterations of soil conditions (Sparling 1992). In our study, the ratio of MBC/SOC was lower in LOP than in NSF stands for both 0-15 cm and 15-30 cm soil depths, suggesting the decreases of organic matter in LOP stands (**Table 2**). The lower MBN/TN and MBP/TP in LOP stands supported the conclusion obtained by Joergensen and Scheu (1999), i.e., the decline of MBC/SOC meant the decrease of available organic matter in soils. These results are consistent with the conclusion of Wang and Wang (2007) who reported that microbial quotient decreased in pure coniferous plantation in the subtropical forest ecosystem.

The two forest types investigated exhibited significant seasonal variation in soil MBC, but not in soil MBN and MBP in Northeast China. In both NSF and LOP stands, MBC concentration was higher in summer, indicating the more immobilization of nutrients by the microbial biomass from the decomposing litters. Furthermore, the soil temperature and moisture were favourable for the microbial growth during the summer (Table 1). This result was similar to the previous studies in temperate forests (Bohlen *et al.* 2001). The result of less sensitive to seasonal changes in MBN and MBP obtained in our study was consistent with Chen et al. (2006) who reported that the seasonal patterns were less obvious in Mongolian pine plantations at the Keerqin sand lands (42º43' N, 122º22' E), China. This is because the potential for N and P immobilization-mineralization by microbial biomass is stable during the growing season in temperate forests.
In summary, the concentrations of MBC, MBN and MBP, and the ratios of MBC/SOC, MBN/TN and MBP/TP reduced significantly in LOP stands; which suggested that the NSF was better to conserve microbial activity than the LOP in Northeast China.

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