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Spatial variability of soil nutrients and microbiological properties after the establishment of leguminous shrub *Caragana microphylla* Lam. plantation on sand dune in the Horqin Sandy Land of Northeast China

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ABSTRACT

The objective of this study is to determine the spatial variability of nutrients, microbial biomass, and enzyme activities of soil due to the establishment of shrub plantation on moving sandy dunes, as part of an effort to understand the microenvironmental factors that control the soil microbiological properties. *Caragana microphylla* Lam., an indigenous leguminous shrub, is the dominant plant species used to control desertification in the semi-arid Horqin Sandy Land of Northeast China. In a 26-year-old *C. microphylla* plantation, soil samples were collected from three soil depths (0–5 cm, 5–10 cm, and 10–20 cm), three slope positions (windward slope, top slope, and leeward slope), and two microsites (under shrubs and between shrubs). The results showed significant differences in soil EC, nutrient content (except for total K), microbial biomass C and N, and the activities of dehydrogenase, urease, and protease at different slopes, soil depths, and microsites. Significant differences in pH at different microsites and slopes, soil moisture and polyphenol oxidase activity at different soil depths and slopes, and activities of phosphomonoesterase and nitrate reductase at different soil depths were also observed. The soil nutrient contents and microbiological activities were greater in the surface soil layer and decreased with the increase of soil depth. Soil organic C, total N, total P, available P and K, microbial biomass C and N, and the activities of enzymes tested (except for protease) under shrubs were higher than those in between shrubs. Furthermore, significant correlations among soil organic C, microbial biomass C and N, the activities of phosphomonoesterase, dehydrogenase, urease, protease, and nitrate reductase were observed, and correlations were also found among EC, total N, total P, available P and K, enzyme (except for polyphenol oxidase) activities, and microbial biomass C and N contents. These results suggest that microenvironmental factors (slope, soil depth and microsite) have significant influences on the spatial distribution of soil nutrients and microbiological properties when the *C. microphylla* sand-fixing plantation is established in the moving sand dunes in the Horqin Sandy Land.

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1. Introduction

Desertification refers to land degradation in arid, semi-arid, or dry sub-humid areas caused by various factors, including climatic variations and human activities (Kassas, 1995). Desertification may lead to the formation of sand dunes (Zhu and Chen, 1994). Sand mobility and wind erosion are the main characteristics of active sand dunes. Vegetation restoration on sand dunes is a major challenge for ecologists (Schade and Hobbie, 2005; Thompson et al., 2005; Zhang et al., 2004, 2006; Su et al., 2005), because the spatial patterns of soil nutrients influence the functioning of individual plants (Antonovics et al., 1987; Gallardo, 2003).

The concentrations of nutrients (e.g., organic C, N, P, and K) are good indicators of soil quality and productivity because of their favorable effects on the physical, chemical, and biological properties of soil (Bauer and Black, 1994; Doran and Parkin, 1994). In addition to nutrient contents, the microbiological and biochemical status of soil has often been used as an early and sensitive indicator of soil ecological stress or restoration processes in both natural and agroecosystems (Badiane et al., 2001). Microbial biomass and enzyme activities of soil are more sensitive than organic carbon concentration for changes in management practices (Powlson et al., 1987; Bergstrom et al., 1998). Soil microbial biomass acts as a reservoir of critical nutrients and is a major determinant for governing the nutrient availability and resource base for nutrient release, which ultimately reflects the soil fertility levels. Soil enzymes play an important role in organic matter decomposition and nutrient cycling, and their activities are related to the soil physical–chemical

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characters (Amador et al., 1997), microbial community structure (Waldrop et al., 2000; Kourtev et al., 2002), vegetation (Waldrop et al., 2000; Sinsabaugh et al., 2002), disturbance (Eivazi and Bayan, 1996; Boerner et al., 2000), and succession (Tscherko et al., 2003).

In arid and semi-arid environments, soil properties are characterized by a high spatial heterogeneity (Austin et al., 2004; Farley and Fitter, 1999; Maestre et al., 2003), and the heterogeneity has been attributed to heterogeneous plant distribution because plants are important in the regulation of soil nutrient availability and distribution by altering the physical, chemical, and biological properties of the soil beneath plant canopies and by concentrating biomass and organic matter (Gross et al., 1995; Augusto et al., 2002; Jackson and Caldwell, 1993a,b; Schlesinger et al., 1996; Zhang and Chen, 2007). For example, Titus et al. (2002) argued that the presence of shrubs strongly influenced microsite soil characteristics and resulted in higher nutrient levels in sandy land. The improvement of soil nutrients by vegetation occurs mainly through the decomposition of organic residues and the absorption of nutrients that have been leached from the surface (Antunes et al., 2008).

The Horqin Sandy Land located in the semi-arid agro-pastoral transitional zone in Inner Mongolia of Northern China (42°41′–45°15′ N, 118°35′–123°30′ E, elevation 180–650 m) is a typical case in China. In the last few decades, the Horqin Sandy Land suffered from severe desertification, and active sand dunes were formed (Liu and Zhao, 1993; Zhu and Chen, 1994). Recently, although the trend of desertification has slowed down, the sandy land area is still expanding. Vegetation restoration is one of the most common and effective ways to combat and control desertification (Su et al., 2005, 2007). Planting indigenous trees, shrubs, and grasses adaptive to sandy lands has been accepted as a common measure west of the Horqin region since the 1980s (Zhu and Chen, 1994; Cao et al., 2000; Zhao et al., 2007).

Among the shrubs planted, a perennial leguminous shrub, *Caragana microphylla* Lam. is the dominant plant species and is widely used as the pioneer species in vegetation reestablishment in the Horqin Sandy Land to stabilize the shifting sand. Large areas of this shrub plantation were artificially established as sand binders on desertified sandy land since the 1980s, which should give rise to significant changes in the local ecosystem. For example, recent studies reported that *C. microphylla* plantations could improve soil water holding capacity, enhance organic carbon and total nitrogen accumulation, and stabilize shifting sands (Su and Zhao, 2003; Cao et al., 2004). Zhang et al. (2006) found that soil properties at the shrubs' individual scales exhibited significant spatial variations in the Horqin Sandy Land, with fine particle fractions, higher water-holding capacity, and lower bulk density. However, little attention has been paid to the spatial variations of soil biochemical and microbiological properties and nutrients at different soil depths, slope positions and microsites in the sand dune, which have important effects on the shrub growth, grass invasion, sand-fixing community development, and nutrient flow in semi-arid ecosystems. Information on the spatial heterogeneity of soil biochemical and ecological properties, as well as nutrients is needed to understand the action range and extent of sand-fixing shrubs, the phytoremediation mechanisms, the interactions between soil and plant community, and the management and conservation of the eco-environment in the shrub plantation.

The objectives of this study are (1) to investigate the spatial variability of soil nutrients, microbial biomass, and enzyme activity after the establishment of *C. microphylla* Lam. plantation on sand dune, (2) to analyze the correlations among them, and (3) to evaluate the contributions of the plantation to improving the soil quality in the stable sand dunes of the Horqin Sandy Land.

2. Materials and methods

2.1. Study area description

The study area is located in the western part of the Horqin Sandy Land at Wulanaodu Village (43°00′ N, 119°39′ E), Wengniute County, Inner Mongolia, China. This village is an experiment and demonstration field site of the Wulanaodu Experimental Station of Desertification, Chinese Academy of Sciences. The Wulanaodu Region belongs to the continental semiarid monsoon climate in the temperate zone, with windy and dry winters and springs, and warm and comparatively rain-rich summers, followed by short and cool autumns. According to the statistics of the Wulanaodu Weather Station, the annual mean temperature is 6.3 °C, and the frost-free period is 130 days. The precipitation is quite irregular from one year to another, and shows strong seasonal variability. The annual mean precipitation is 340.5 mm, with 70–80% of the precipitation occurring between May and September, and the annual mean pan-evaporation is around 2500 mm. The average annual wind speed ranges from 3.2 to 4.5 m s^{-1}, with most of the windy days and wind storms occurring between March and May. The surface sand deposits are 20–120 m thick (Zhang et al., 2004). The soils are classified as cambic arenosols (FAO, 2006), sandy in texture, light yellow in color, and low in organic matter content. Due to these characteristics, the soil is particularly susceptible to wind erosion. Due to cultivation, overgrazing, windy climate, and loose sandy soils land degradation in this region has spread rapidly. The landscape is characterized as a mosaic of mobile sand dunes, semi-fixed dunes, stabilized dunes, and lowland meadows (Zhao et al., 2000). The original vegetation is grassland (dominant species include *Stipa grandis*, *Aneurolepidium chinense*, and *Agropyron cristatum*) with sparsely scattered woods (mainly *Ulmus pumila*). However, the original vegetation has been greatly altered over the past several decades, primarily due to long-term overgrazing and overcutting. The sandy land vegetation is generally dominated by psammophytes and some shrubs (e.g., *C. microphylla*, *Atraphaxis manshurica*, and *Salix gordejevii*), semi-shrubs (e.g., *Artemisia halodendron* and *Artemisia frigida*), forbs (e.g., *Agriophyllum squarrosum*, *Salsola collina*, *Lespedeza davurica*, and *Artemisia scoparia*), and grasses (e.g., *Aristida adscensionis*, *Calamagrostis chinesis*, *Digitaria ciliaris*, *A. chinense*, and *Pennisetum flaecidum*).

There is a large area of *C. microphylla* plantation in the Wulanaodu Region. *C. microphylla* was gradually planted on the desertified sandy land with the help of straw checkerboards as sand binders since 1983. The checkerboards were composed of 1 m × 1 m squares made of straw. Before planting, the dominant plant species on the desertified sandy land was *A. squarrosum*, and the vegetative cover was generally less than 5%. *C. microphylla* grew to form 1 m-high shrubby belts 3–5 years after planting. The experimental site was enclosed after seeding and the oldest plantation is now 26 years old.

The research was carried out in a stable sand dune with 26-year-old *C. microphylla* plantation (row and plant spacing 1 m × 1 m). The sand dune is running along the direction of northwest–southeast, and the length, width and height of slopes are about 200, 60, and 25 m, respectively. The mean height, shoot number and crown diameter of *C. microphylla* are 85.0 cm, 14.5, and 65 cm × 60 cm, respectively. In growing season, some annual plants (e.g., *S. collina*, *Bassia dasyphylla*, *Corispermum thelegium*, *Chenodium acuminatum* and *Setaria viridis*) randomly distribute in the glade and under the shrub crown, and the vegetative cover is less than 10%.

2.2. Experiment design and soil sampling

In our experiment, three slope positions (middle part of windward slope, top slope, and leeward slope) of the sand dune with 26-year-old *C. microphylla* plantation were selected as the experimental sites. The sampling period was in April 2009, the beginning of the growing season. The soil environment was relatively stable and was seldom affected by wind and other factors. On each slope, two microsites (under shrub lands-US, and between shrub lands-BS) with the same growth conditions were chosen as sampling plots. Four soil samples, 20 cm from the center of the shrub or 20 cm from the edge of shrub, were collected in four directions, and mixed as a pooled sample. Four transects as four replications, each size 10 m × 10 m, and each 10 m apart, were run through the sand dune system. The soil samples were taken from depths of 0–5, 5–10, and 10–20 cm, respectively. The samples at the depth of 0–5 cm were collected by shovel, and those at the depth of 5–20 cm were collected using a 5 cm-diameter soil auger. A total of 72 soil samples (3 slopes × 2 microsites × 3 soil depths × 4 replicates) were collected. All samples were sieved using a 2 mm screen, and the roots and other debris were removed and discarded. Half of each sample was kept field-moist in a cooler at 4 °C, and the other half was air-dried and stored at room temperature. The field-moist samples were analyzed four weeks after sampling.

2.3. Laboratory analysis

The soil pH and electrical conductivity (EC) were measured in a soil–water suspension (1:1 and 1:5 soil–water ratios, respectively). Part of the air-dried and sieved samples was ground and passed through a 0.25 mm screen for soil organic carbon (SOC) and total N analyses. SOC was measured by the $K_2Cr_2O_7$–H_2SO_4 oxidation method of Nelson and Sommers (1982), and the total nitrogen was determined by the semimicro-Kjedahl digestion method (Nelson and Sommers, 1982). The soil total P and available P were determined by the Olsen and Dean method (ISSCAS, 1978), and the total K and available K were measured by atomic absorption spectroscopy (ISSCAS, 1978).

Soil microbial biomass C and N were estimated by the chloroform fumigation-incubation method (Jenkinson and Powlson, 1976) with some modifications. Briefly, the soil (25 g fresh weight) was weighed in glass scintillation vials. The vials were placed in a desiccator with a wet paper towel and a 50 mL beaker containing 40 mL of ethanol-free chloroform and a few glass beads. The desiccator was evacuated, and the soil was exposed to chloroform vapors for 24 h. After fumigation, an inoculum was added, and the soil was adjusted to about 55% water holding capacity. Then, the soil was incubated with 10 mL 1 M NaOH at 25 °C for 10 days in darkness. The CO_2-C trapped in the alkali was determined by titration. After CO_2 sampling, NH_4^+-N was extracted with 100 mL 2 M KCl and quantified by steam distillation (Soil Science Society of China, 2000).

The soil urease activity was measured using urea as the substrate, and the released ammonium was assayed colorimetrically at 460 nm (Kandeler and Gerber, 1988). The dehydrogenase activity was determined by the reduction of triphenyltetrazolium chloride (TTC) to triphenyl formazone (TPF). Briefly, 5 g soil was incubated in 5 mL 5 g L^{-1} TTC and 2 mL 0.1 M glucose at 37 °C for 12 h. The reactions were terminated with 0.25 mL 98% H_2SO_4, and the products were extracted for 30 min with 5 mL toluene using a shaker. After centrifugation, the TPF dissolved in toluene was assayed at 492 nm. The activity was measured following a modified method of the ISSCAS (1985), and expressed as the mg of the TPF released kg^{-1} soil h^{-1}.

The protease activity was determined as reported by Ladd and Butler (1972) with some modifications. Briefly, 2 g of the soil samples was incubated for 2 h in 5 mL of a buffered casein solution (pH 8.1) and 5 mL of TRIS buffer (50 mM, pH 8.1) at 50 °C. The released aromatic amino acids were extracted with trichloroacetic acid (0.92 M) and were measured colorimetrically using a Folin-Ciocalteu reagent. The activity was expressed as mg Tyr (tyrosine equivalents) kg^{-1} soil h^{-1}.

The nitrate reductase activity was determined by colorimetric method (Schinner et al., 1996). Triplicates of 5 g soil samples were incubated with 4 mL of 2,4-dinitrophenol solution (0.9 mM), 1 mL potassium nitrate solution (25 mM), and 5 mL distilled water at 25 °C for 24 h. The control sample was incubated at −20 °C for 24 h. Then, 10 mL 4 M KCl solution was added to all soil samples, including the control. Then, 5 mL of the filtrate, 3 mL of NH_4Cl buffer (0.19 M, pH 8.5), and 2 mL of color reagent were added. This was left for 15 min for color development. The optical density was observed in a spectrophotometer against the blank at 520 nm. The enzyme activity was calculated using a standard curve.

Polyphenol oxidase activity was measured using the method described by Perucci et al. (2000), and expressed as millimoles of catechol oxidized kg^{-1} soil h^{-1} (on dry weight basis). Alkaline phosphomonoesterase activity was determined as described by Sardans and Peñuelas (2005). Briefly, the soil samples (1 g d.w.) were mixed with 1 mL disodium p-nitrophenyl phosphate solution (115 mM) and 4 ml of THAM (Tris–hydroxymethyl-aminomethane, with the acids citric, maleic and boric) buffer solution (pH 11.0) and incubated at 37 °C for 1 h. The p-nitrophenol released by phosphatase activity was extracted and colored with NaOH and determined photometrically at 400 nm. Phosphomonoesterase activity was expressed as mg p-nitrophenol per kilogram dry matter and incubation time.

2.4. Statistical analysis

All results are reported as means ± standard deviations. All the data were analyzed by three-way ANOVA using slope, microsite, and soil depth as factors. Analyses of variance (ANOVA) and multiple comparisons were used to determine the differences among the treatments. Pearson correlation coefficients were used to evaluate the relationships between the corresponding variables. All statistical analyses were performed using the SPSS software package. A difference at $P < 0.05$ level was considered as statistically significant.

3. Results

3.1. Spatial variability of soil moisture, pH, and electrical conductivity

The soil moisture varied significantly with slope and soil depth, and the pH varied significantly with slope, as well as microsite ($P < 0.05$) (Table 1). The pH among the soil depths and the soil moisture between the two microsites showed no significant differences. The pH values (0–5, 5–10, and 10–20 cm) in the US of three slopes were all lower than those in the BS, respectively (Table 2). Soil moisture and electrical conductivity (EC) decreased sharply with the increase of soil depth at three slope positions. The soil moisture in each depth of BS of the three slopes was higher than that of US. According to data in Table 2, the effect of depth on soil moisture was more significant than that of the slope. Soil EC values in the 26-year-old *C. microphylla* plantation ranged from 18.94 μS cm^{-1} to 114.63 μS cm^{-1} and varied significantly with slope, soil depth, and microsite. The variations of EC

Table 1
Results of analysis of variance for soil pH, moisture, nutrients, microbial biomass and enzyme activities in different slopes, different soil depths and different microsites.

Index	Slope		Microsite		Soil depth	
	F	*P*	*F*	*P*	*F*	*P*
pH	7.061	0.003	14.070	0.001	3.036	0.060
Moisture content	4.507	0.018	2.970	0.093	108.189	<0.001
Electrical conductivity	10.181	0.001	5.853	0.023	62.155	<0.001
Total N	13.773	<0.001	10.201	0.003	119.803	<0.001
Organic matter	20.685	<0.001	7.329	0.001	87.131	<0.001
Total P	12.033	<0.001	31.291	<0.001	53.137	<0.001
Total K	1.390	0.112	2.072	0.153	1.970	0.154
Available P	9.275	0.001	3.932	0.045	8.112	0.001
Available K	18.823	<0.001	0.032	0.858	101.655	<0.001
Microbial biomass C	6.101	0.016	74.387	<0.001	112.991	<0.001
Microbial biomass N	124.427	<0.001	116.408	<0.001	195.541	<0.001
Polyphenol oxidase	7.062	0.003	0.024	0.879	16.677	<0.001
Phosphomonoesterase	9.041	0.001	2.278	0.140	26.741	<0.001
Dehydrogenase	15.456	<0.001	31.357	<0.001	94.827	<0.001
Urease	10.930	<0.001	10.912	0.002	77.235	<0.001
Protease	11.701	<0.001	25.349	<0.001	63.234	<0.001
Nitrate reductase	1.938	0.159	7.711	0.009	106.819	<0.001

F and *P* values, from three-way ANOVA are given.

Table 2
Soil pH, moisture, EC and nutrients in different slopes, soil depths and microsites (mean ± SD).

Index	Soil depth (cm)	Windward slope		Top slope		Leeward slope	
		US	BS	US	BS	US	BS
pH	0–5	6.38 ± 0.21	7.25 ± 0.08	6.93 ± 0.07	7.03 ± 0.08	7.06 ± 0.13	7.20 ± 0.09
	5–10	6.43 ± 0.42	6.66 ± 0.33	6.71 ± 0.11	6.97 ± 0.07	6.84 ± 0.07	6.88 ± 0.26
	10–20	6.49 ± 0.06	7.07 ± 0.10	6.65 ± 0.10	7.00 ± 0.09	6.78 ± 0.21	7.03 ± 0.20
	US/BS	0.92b		0.97a		0.98a	
Moisture content (%)	0–5	1.61 ± 0.22	2.51 ± 0.33	1.70 ± 0.22	1.80 ± 0.35	1.60 ± 0.13	2.04 ± 0.55
	5–10	0.74 ± 0.23	0.95 ± 0.61	0.45 ± 0.05	0.53 ± 0.14	0.86 ± 0.15	1.24 ± 0.19
	10–20	0.64 ± 0.08	0.76 ± 0.18	0.60 ± 0.05	0.81 ± 0.03	0.78 ± 0.07	0.80 ± 0.18
	US/BS	0.75b		0.84a		0.82a	
Electrical conductivity ($\mu S\,cm^{-1}$)	0–5	114.63 ± 28.57	103.19 ± 21.23	83.95 ± 13.43	80.64 ± 20.43	91.22 ± 17.59	43.94 ± 8.45
	5–10	65.81 ± 33.00	63.54 ± 5.47	40.44 ± 5.87	39.91 ± 11.76	47.32 ± 8.11	25.16 ± 2.52
	10–20	28.04 ± 5.14	25.41 ± 6.24	29.90 ± 4.87	27.83 ± 2.31	30.11 ± 1.41	18.94 ± 1.09
	US/BS	1.08b		1.04b		1.85a	
Total N ($g\,kg^{-1}$)	0–5	0.075 ± 0.009	0.062 ± 0.010	0.071 ± 0.016	0.065 ± 0.014	0.058 ± 0.017	0.044 ± 0.009
	5–10	0.048 ± 0.008	0.037 ± 0.006	0.032 ± 0.011	0.029 ± 0.010	0.033 ± 0.005	0.023 ± 0.007
	10–20	0.015 ± 0.003	0.013 ± 0.005	0.015 ± 0.004	0.017 ± 0.003	0.012 ± 0.006	0.010 ± 0.004
	US/BS	1.22a		1.03b		1.32a	
Organic carbon ($g\,kg^{-1}$)	0–5	1.62 ± 0.077	1.32 ± 0.091	0.90 ± 0.21	0.86 ± 0.11	1.37 ± 0.14	0.95 ± 0.19
	5–10	1.25 ± 0.19	1.15 ± 0.46	0.55 ± 0.27	0.45 ± 0.18	0.99 ± 0.17	0.56 ± 0.10
	10–20	0.42 ± 0.11	0.33 ± 0.10	0.21 ± 0.13	0.12 ± 0.064	0.35 ± 0.13	0.16 ± 0.031
	US/BS	1.20b		1.34b		1.80a	
Total P ($g\,kg^{-1}$)	0–5	1.65 ± 0.14	1.21 ± 0.28	1.60 ± 0.04	0.73 ± 0.25	1.00 ± 0.15	0.80 ± 0.06
	5–10	0.87 ± 0.23	0.68 ± 0.08	0.82 ± 0.02	0.63 ± 0.20	0.80 ± 0.06	0.66 ± 0.14
	10–20	0.55 ± 0.27	0.53 ± 0.07	0.57 ± 0.05	0.54 ± 0.04	0.55 ± 0.03	0.50 ± 0.07
	US/BS	1.23b		1.52a		1.19b	
Total K ($g\,kg^{-1}$)	0–5	2.35 ± 0.02	2.22 ± 0.09	2.15 ± 0.28	2.36 ± 0.04	1.96 ± 0.05	2.17 ± 0.03
	5–10	2.34 ± 0.01	2.25 ± 0.04	2.31 ± 0.04	2.41 ± 0.06	1.96 ± 0.21	2.33 ± 0.02
	10–20	2.30 ± 0.05	2.13 ± 0.07	2.29 ± 0.03	2.25 ± 0.17	2.11 ± 0.03	2.38 ± 0.03
	US/BS	1.06a		0.96a		0.88a	
Available P ($mg\,kg^{-1}$)	0–5	0.18 ± 0.011	0.17 ± 0.019	0.08 ± 0.031	0.06 ± 0.013	0.16 ± 0.041	0.11 ± 0.018
	5–10	0.15 ± 0.056	0.11 ± 0.018	0.13 ± 0.095	0.07 ± 0.058	0.13 ± 0.053	0.07 ± 0.017
	10–20	0.08 ± 0.003	0.08 ± 0.009	0.07 ± 0.028	0.04 ± 0.018	0.12 ± 0.017	0.05 ± 0.013
	US/BS	1.14c		1.65b		1.90a	
Available K ($mg\,kg^{-1}$)	0–5	0.30 ± 0.026	0.29 ± 0.017	0.29 ± 0.019	0.29 ± 0.030	0.27 ± 0.056	0.18 ± 0.020
	5–10	0.22 ± 0.060	0.15 ± 0.046	0.16 ± 0.033	0.16 ± 0.022	0.13 ± 0.028	0.11 ± 0.025
	10–20	0.16 ± 0.018	0.12 ± 0.041	0.14 ± 0.012	0.13 ± 0.025	0.12 ± 0.014	0.09 ± 0.028
	US/BS	1.28a		1.03b		1.34a	

Means in row followed by the different letter are significantly different ($P < 0.05$).

in depth and in microsite followed trends similar to those of soil moisture.

3.2. Spatial variability of soil nutrients

The establishment of *C. microphylla* plantation resulted in an enrichment and spatial variation of organic C, total N, total P, available P, and available K in desertified sandy soils. Significant differences were observed for soil organic carbon, total N, total P, and available P among slopes, soil depths, and between microsites. The differences of total K among the three treatments and the available K between the two microsites were not significant. Significant differences in the available K among the soil depths and slopes were also found (Table 1, $P < 0.01$). The organic C, total N, total P, available P, and available K decreased with increasing soil depth, and the contents in each soil depth (0–5 cm, 5–10 cm, and 10–20 cm) were significantly higher in US than those in BS, with enrichment ratios (US/BS) of 1.20–1.80, 1.03–1.32, 1.19–1.52, 1.14–1.90, and 1.03–1.34, respectively (Table 2).

3.3. Spatial variability of soil microbial biomass

The soil microbial biomass C (MBC) and N (MBN) differed significantly among different slopes, different soil depths, and between the two microsites (Table 1). Both MBC and MBN of soil in the windward, top, and leeward slopes were much greater in the surface soil (0–5 cm) than those in the subsurface soil, showing a consistent decrease with the increase in soil depth, especially for MBC from the 0–5 cm to 5–10 cm soil layers (Fig. 1). The MBC in the 0–5 cm soil layer in the windward, top, and leeward slops was increased 1.26-, 2.39-, and 1.66-fold and by 1.96-, 3.12-, and 2.91-fold, respectively, compared with those in the 5–10 and 10–20 cm layers, and the MBN was increased 1.19-, 1.34-, and 1.16-fold and 1.37-, 2.38-, and 1.42-fold, respectively. These results suggested that the effect of depth on MBC was more significant than that on MBN. The MBC and MBN in the 0–5, 5–10, and 10–20 cm soil layers of US were all higher than those in BS, and the enrichments ratios (US/BS) were 1.58–1.94 and 1.16–1.32, respectively. Overall, the variations of MBN and MBC with slope, soil depth and microsite followed trends similar to those of total N, organic C, total P, available P, and available K.

3.4. Spatial variability of soil enzyme activity

The values of soil polyphenol oxidase, phosphomonoesterase, dehydrogenase, urease, protease, and nitrate reductase activities at different soil depths (0–5, 5–10, and 10–20 cm) in each slope are shown in Table 3. Significant differences in protease, urease, and dehydrogenase activities among slopes, between the two microsites, and among soil depths were found; differences in the activities of polyphenol oxidase and phosphomonoesterase among slopes and soil depths, and the differences of nitrate reductase activity between the two microsites, and among soil depths were also observed (Table 1). The activities of soil phosphomonoesterase, dehydrogenase, urease, protease, and nitrate reductase of all the treatments were higher in the topsoil layer (0–5 cm) and significantly decreased with depth, whereas the activity of soil polyphenol oxidase in the 5–10 cm layer was higher than those in the other two soil depths. With the exception of protease, in each slope and soil depth, the other five enzyme activities in US were all higher than those in BS, especially in the 0–5 cm depth. The enrichment ratios of US/BS of polyphenol oxidase, phosphomonoesterase, dehydrogenase, urease, and nitrate reductase activities were 1.18–1.40, 1.16–1.43, 1.62–2.27, 1.14–1.61, and 1.80–2.15, respectively. The variations of enzyme activities with the slope differed with the enzyme type. For example, polyphenol oxidase and dehydrogenase activities in the top slope were higher than those in the windward and leeward slopes, while phosphomonoesterase activities showed windward slope > leeward slope > top slope.

3.5. Correlation coefficients among soil pH, EC, nutrients, microbial biomass, and enzyme activity

The correlation coefficients among soil pH, EC, nutrients, microbial biomass, and enzyme activity in the stable sand dune with *C. microphylla* plantation are shown in Table 4. There were significant correlations among soil organic carbon, MBC, MBN, phosphomonoesterase, dehydrogenase, urease, protease, and nitrate reductase activities. Positive and significant correlations were also found among EC, total N, total P, available P and K, the enzyme (except for polyphenol oxidase) activities, MBC, and MBN. Moreover, polyphenol oxidase activities were correlated negatively and significantly with the total K, organic C, and the activities of protease and nitrate reductase. The pH and total K are not correlated to soil nutrients, enzyme activities, and microbial biomass.

4. Discussion

The previous study and our present results indicate that establishing *C. microphylla* on desertified sandy land results not only in vegetation restoration and conversion from moving or semi-moving sand dune to fixed sand dune (Cao et al., 2000, 2008), but in the significant spatial variability of soil physicochemical and microbiological properties. *C. microphylla* shrubs function as natural barriers to reduce wind velocity. Consequently, plantation establishment led to the collection of windblown fine materials by entrapment and deposition of dust through stemflow and throughfall. The difference in wind speed at different slopes results in the spatially heterogeneous distribution of fine particle dust rich in nutrients falling at different positions of the sand dune and depositing in the surface soils under plant canopies. The topography in a sand dune, from the windward slope to the leeward slope, affects the soil moisture content, SOC, and total N, and partly contributes to the spatial distribution of soil chemical and microbiological properties. These are probably the main reasons why the soil nutrients and microbial biomass were higher on windward slope.

The significant differences in the spatial variation of soil moisture, EC, and pH were mainly observed at different soil depths, and between the two microsites. In this study, however, the increase in EC due to shrub establishment did not necessarily result in an increase in soil salinity because the EC values were relatively low. The EC values correlated with the SOC content and probably reflected an enhancement in soil nutrient concentrations released by litter decomposition. In natural ecosystems, the litter on the soil surface and the turnover of fine roots are regarded as the main pathways of SOC input. Due to the higher inputs of organic material at the soil surface, the SOC level generally declined with soil depth. Nitrogen-fixing legumes have been shown to increase soil N levels. Nitrogen fixation by *C. microphylla* and N release by litter decomposition resulted in an increase in soil N content. The annual herbs in the plantation play an important role in sandy land productivity, and their rapid growth and death provided an important influx of SOC, N, and other nutrients (Cao et al., 2000, 2004, 2008). The differences in species and the quantity of herbs distributed on the slopes also resulted in the uneven spatial distribution of soil nutrients, microbial biomass, and enzymes activities. The change

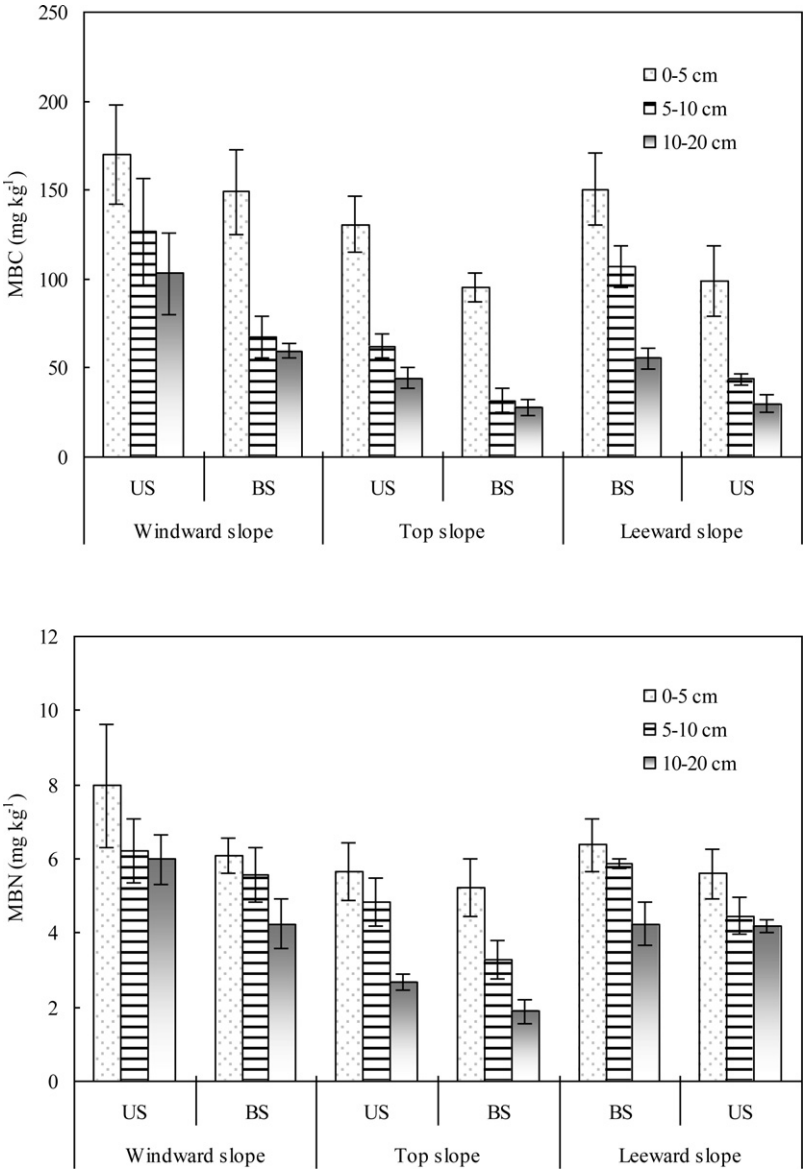

Fig. 1. Soil microbial biomass C (MBC) and N (MBN) in different slopes, soil depths and microsites (mean ± SD).

in soil properties reflects the variations in the microenvironment at different slope positions.

In the area beneath the plants, the soil conditions are better compared with the soils between shrubs (Wezel et al., 2000; Dong et al., 2009). Moreover, the root architecture of shrubs plays a key role in affecting soil nutrients, microbial biomass contents, and enzyme activities (Hansson et al., 1995; Dong et al., 2009). The number and biomass of the roots affected the distribution range of soil nutrient and microbiological properties. In our study, SOC, total N, total P, available P and K, MBC, MBN, and activities of enzyme (except for protease) in US were higher than those in BS, possibly due to the presence of more roots, as well as the accumulation of SOC and soil nitrogen in US. In many arid and semi-arid ecosystems, canopy shrubs have a strong positive influence on soil moisture and nutrient availability, creating islands of fertility where organic matter and nutrients are high compared with the areas outside the canopy (Schade and Hobbie, 2005; Su et al., 2002). *C. microphylla* enriched the C, N, P, K, MBC, and MBN under their canopy, and the typical "fertile islands" phenomenon was also observed in this study.

Our results also confirm that nutrient content and microbial activities are greater in the surface soil layer than in the deeper layers. This is attributed to the improved soil environment and increased organic and inorganic materials released from plants, which provide the energy source and nutrients for microorganisms largely entering the soil through the surface. The activities of the analyzed enzymes decreased with increasing soil depth, confirming previous observations (Sardans and Peñuelas, 2005; Chen, 2003). The asymmetrical nutrient input through the soil profile due to the aboveground litter incorporation on the soil surface, as well as better soil aeration account for the higher soil enzyme activity in the topsoil than that in the lower depths. Decreases in soil enzyme activities through the soil profile have been observed in forest and agricultural soils when there are organic inputs to the soil surface (Aon et al., 2001; Taylor et al., 2002; Chen, 2003; Cao et al., 2008).

The type and amount of organic matter affects the biodiversity of soil microflora and microbe distribution, thus accounting for the changes in enzyme activity distribution (Sinsabaugh et al., 2002). Given that microorganisms are the main agents of organic matter transformation and the main sources of enzymes in the soil, a

Table 3
Soil enzymes in different slopes, soil depths and microsites (mean ± SD).

Index	Soil depth (cm)	Windward slope		Top slope		Leeward slope	
		US	BS	US	BS	BS	US
Polyphenol oxidase (mmol kg^{-1} soil h^{-1})	0–5	1.91 ± 0.396	1.37 ± 0.452	1.92 ± 0.498	1.20 ± 0.307	1.18 ± 0.378	1.11 ± 0.308
	5–10	2.63 ± 0.123	2.05 ± 0.934	4.08 ± 0.878	2.83 ± 0.804	2.29 ± 0.470	1.70 ± 0.920
	10–20	1.94 ± 0.255	1.88 ± 0.250	2.27 ± 0.486	1.98 ± 0.373	1.86 ± 0.234	1.65 ± 0.318
	US/BS	1.24b		1.40a		1.18b	
Phosphomonoesterase (mg kg^{-1} soil h^{-1})	0–5	12.27 ± 2.70	8.81 ± 3.08	6.53 ± 2.09	5.66 ± 2.56	7.79 ± 1.19	6.95 ± 1.76
	5–10	7.70 ± 3.24	5.48 ± 2.58	3.61 ± 1.54	3.42 ± 0.23	6.44 ± 1.67	5.89 ± 0.97
	10–20	3.23 ± 0.41	3.07 ± 0.68	1.44 ± 0.65	1.14 ± 0.83	3.92 ± 0.85	1.89 ± 0.60
	US/BS	1.28b		1.16b		1.43a	
Dehydrogenase (mg TPF kg^{-1} soil h^{-1})	0–5	11.25 ± 1.87	2.63 ± 1.67	15.67 ± 3.11	9.22 ± 0.64	5.87 ± 1.86	2.31 ± 0.87
	5–10	0.44 ± 0.07	0.42 ± 0.30	1.01 ± 1.73	0.50 ± 0.05	1.27 ± 1.10	0.41 ± 0.03
	10–20	0.39 ± 0.04	0.38 ± 0.40	0.44 ± 0.02	0.39 ± 0.02	0.45 ± 0.07	0.38 ± 0.04
	US/BS	2.12a		1.62b		2.27a	
Urease (mg NH_4^+-N kg^{-1} soil h^{-1})	0–5	74.14 ± 8.93	68.83 ± 17.54	55.33 ± 7.40	34.78 ± 3.75	68.72 ± 10.22	42.40 ± 8.12
	5–10	49.42 ± 7.18	44.69 ± 5.95	35.73 ± 7.34	22.57 ± 2.58	47.55 ± 6.56	29.36 ± 5.07
	10–20	18.38 ± 5.00	14.87 ± 0.35	15.05 ± 3.32	9.03 ± 2.16	15.10 ± 3.31	11.47 ± 2.34
	US/BS	1.14b		1.61a		1.52a	
Protease (mg Tyr kg^{-1} soil h^{-1})	0–5	19.17 ± 3.45	27.96 ± 4.46	20.09 ± 3.55	22.07 ± 3.13	24.09 ± 2.37	31.48 ± 4.60
	5–10	8.97 ± 2.21	14.77 ± 1.40	7.78 ± 1.87	15.16 ± 2.65	17.92 ± 2.19	25.57 ± 3.13
	10–20	3.63 ± 0.67	8.87 ± 1.68	4.05 ± 1.02	10.29 ± 1.92	7.62 ± 0.88	11.48 ± 2.05
	US/BS	0.57a		0.61a		0.71a	
Nitrate reductase (mg kg^{-1})	0–5	4.40 ± 1.17	3.77 ± 0.85	3.40 ± 0.21	2.20 ± 0.65	4.34 ± 0.67	2.83 ± 0.43
	5–10	1.06 ± 0.15	0.53 ± 0.04	0.93 ± 0.07	0.36 ± 0.08	0.95 ± 0.17	0.45 ± 0.07
	10–20	0.11 ± 0.02	0.04 ± 0.01	0.07 ± 0.01	0.03 ± 0.01	0.07 ± 0.02	0.04 ± 0.01
	US/BS	1.97ab		2.15a		1.80b	

Means in row followed by the different letter are significantly different ($P < 0.05$).

Table 4
Correlation coefficients among soil pH, EC, nutrients, microbial biomass and enzyme activity in the stable sand dune with *C. microphylla* plantation ($n = 72$).

	pH	EC	TN	TP	AVP	TK	AVK	SOC	POA	PHA	DHA	UA	PRA	NRA	MBC	MBN
pH	1															
EC	0.01	1														
TN	−0.03	0.88**	1													
TP	−0.11	0.65**	0.78**	1												
AVP	−0.07	0.54**	0.42**	0.45**	1											
TK	−0.21	−0.16	−0.16	−0.08	−0.16	1										
AVK	0.19	0.88**	0.86**	0.69**	0.39**	−0.13	1									
SOC	−0.04	0.78**	0.70**	0.52**	0.57**	−0.20	0.71**	1								
POA	−0.09	−0.16	−0.19	−0.11	−0.13	0.38**	−0.19	−0.31*	1							
PHA	−0.03	0.69**	0.53**	0.36**	0.46**	−0.06	0.56**	0.76**	−0.06	1						
DHA	−0.04	0.60**	0.81**	0.83**	0.12	−0.10	0.65**	0.45**	−0.18	0.38**	1					
UA	−0.01	0.75**	0.75**	0.54**	0.55**	−0.18	0.69**	0.87**	−0.18	0.73**	0.55**	1				
PRA	0.32	0.39**	0.43**	0.24	0.32*	−0.01	0.45**	0.57**	−0.33*	0.49**	0.37**	0.60**	1			
NRA	0.13	0.79**	0.76**	0.61**	0.43**	−0.20	0.74**	0.73**	−0.33*	0.61**	0.66**	0.78**	0.54**	1		
MBC	−0.09	0.61**	0.60**	0.62**	0.38**	−0.11	0.57**	0.55**	−0.21	0.46**	0.55**	0.62**	0.36**	0.74**	1	
MBN	−0.25	0.68**	0.68**	0.57**	0.49**	−0.11	0.62**	0.76**	−0.18	0.57**	0.50**	0.74**	0.29*	0.63**	0.71**	1

EC: electrical conductivity; TN: total N; TP: total P; AVP: available P; TK: total K; AVK: available K; SOC: soil organic matter; POA: polyphenol oxidase; PHA: phosphomonoesterase; DHA: dehydrogenase; UA: urease; PRA: protease; NRA: nitrate reductase; MBC: microbial biomass C; and MBN: microbial biomass N.

* $P < 0.05$.

** $P < 0.01$.

higher microbial biomass and higher enzyme activities should be expected when the organic matter content is higher. This could explain the significantly positive relationships among soil organic carbon, microbial biomass, and enzyme activities in this study. As consequences of higher soil organic matter content at surface depth, windward slope, and US, the soil microbial biomass and enzyme activities were also higher.

5. Conclusions

The results of the present study indicate that the establishment of leguminous shrub *C. microphylla* plantation on the semi-arid Horqin Sand Land resulted in the spatial variability of soil nutrients and microbiological properties, which was manifested in the significant differences in soil EC, nutrient content (except for total K), microbial biomass C and N, and the activities of dehydrogenase, urease, and protease at different slopes, soil depths, and microsites. The soil nutrient contents and microbiological activities were all greater in the surface soil layer and decreased with the increase of soil depth. The soil nutrients, microbial biomass, and enzyme activities (except for protease) under shrubs were higher than those between shrubs. The spatial variability of soil nutrient contents was correlated to that of microbiological properties in the sandy land. Our results confirm that the establishment of shrub plantation and

microenvironmental factors (slope, soil depth, and microsite) have significant influences on the spatial distribution of soil nutrients and microbiological properties in sand dune.

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