Effect of soil sealing on the microbial biomass, N transformation and related enzyme activities at various depths of soils in urban area of Beijing, China

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Abstract

Purpose Sealing of soils prevents the exchange of gas, water and nutrient between soil and other environmental compartments, and affects urban N flux, thereby resulting in certain negative impacts on soil functioning and urban environment. However, little information is available on the biogeochemical cycling and biological activities after sealing of soils in urban areas. The aim of this study was to assess the effects of soil sealing on N transformation and associated microbial properties.

Materials and methods Soil samples were collected at four depths (0–10, 10–20, 20–30, 30–40 cm) from three land cover types: impervious land (sealing of soil), forest (vegetation) and bare land (no sealing and no vegetation) in Beijing, China. Soil inorganic N, net potential N-mineralization and nitrification rates, physicochemical properties, microbial biomass and activities of urease, protease and nitrate reductase were analyzed.

Results and discussion Sealing of soils affected soil physicochemical properties, nutrient availability and microbial characterizations, which resulted in the reduction of soil net potential mineralization and nitrification. Soil pH and

C/N ratio were increased while soil organic matter and total N were decreased after sealing of soils, especially in the 0–10 cm layer. Impervious sites had the lowest soil microbial biomass C (10.52–26.26 mg kg⁻¹) and N (10.52–26.26 mg kg⁻¹) among the three types of soils. In addition, microbial biomass increased along the depth gradient. Soil enzyme activities were also significantly affected by sealing of soils. Canonical correlation analysis suggested that reduction of soil net potential mineralization and nitrification in impervious sites was mainly caused by the variation of soil total N, soil organic matter and soil microbial biomass. **Conclusions** Sealing of soils remarkably influenced the soil N transformation process and soil microbial activities in urban areas. Thus, our results suggested that reducing the negative effects of soil sealing on paved and unpaved areas could be useful measures to improve urban soil functions, and hence facilitate the remediation of the adverse impacts caused by urban N deposition.

Keywords Enzyme activity · N mineralization · Sealing of soils · Soil microbial biomass · Urban

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1 Introduction

The rapidly expanding urban areas and intensive human activities significantly affect the ecosystem services and living environment, which has become an increasing concern in recent years (Daily 1997; Daily et al. 2002, 2009; Wang et al. 2010a, b). Due to urbanization, the original landscape dominated by natural vegetation was gradually replaced by numerous buildings and concrete surfaces (Li et al. 2005, 2011). Sealing of soils, here described as the covering by impervious materials, e.g., asphalt, concrete, plastic and glass, is the main characterization of urbanization

and changes of urban land cover (Scalenghe and Marsan 2009). Sealing of soils prevents the exchange of material and energy between soil and other environmental compartments, thereby resulting in certain negative impacts on soil functioning and urban environment (EEA 2001; Zhao et al. 2010). Most previous studies have focused on the effects of expanding sealed areas on heat island (Brundl and Hoppe 1984; Takebayashi and Moriyama 2007), water runoff (Baumhardt et al. 1990; Bhaduri et al. 2001; Peffy and Nawaz 2008), gas diffusion (Wiegand and Schott 1999; Smagin 2000; Kaye et al. 2004) and biodiversity (Savard et al. 2000; Pauchard et al. 2006) in urban ecosystem, but the information about the quality of soils underneath the sealed areas, and the effects of soil sealing on biogeochemical cycling, in particular, nutrient cycle and biological component in urban areas is limited. However, soils underneath the sealed areas were still carrying out important functions, e.g., supporting the growth of roadside trees, allowed the infiltration of storm-water (Burghardt et al. 2004; Burghardt 2006; Gundula et al. 2011). Therefore, investigation and quantitative evaluation the properties and processes of the soils underneath the sealed areas is essential to soil protection, management and restoration (Karlen et al. 2001; Karlen et al. 2003; EC 2006).

The holistic determination of soil nutrient dynamics and microbial properties is an efficient measure to assess the quality and function of soils. N is a key nutrient required by all living organisms, which controlling the species composition, diversity, dynamics and function of many terrestrial ecosystems (Vitousek et al. 1997; Chen et al. 2010; Geisseler et al. 2010). However, urban soil sealing alternated the biotic and abiotic factors that regulating N transformation and seriously affected soil N pool and flux (Lorenz and Lal 2009). This, in turn, would influence nutrient availability of soil organisms and roadside tree growth (Jim 1987, 1998; Scharenbroch et al. 2005). Moreover, researchers suggest that leaching of excessive inorganic nitrogen is the main factor that causes urban groundwater pollution and affects human health (Fleige et al. 1980; Mitchell et al. 2005). Thus, investigation of N transformation, inorganic N distribution and the relevant soil properties underneath sealed areas is urgently needed to assess the potential risk of N losses and other negative consequences for urban ecosystem. In addition, soil nitrogen transformation is microbial mediated and microbial properties has been used frequently for evaluating soil nutrient status and functions (Pascual et al. 1999; Critter et al. 2004; Stark et al. 2008). Among them, soil microbial biomass has been called “the eye of the needle through which all the nature organic material that enter the soil must pass” and its turnover is a key process to maintain N cycles (Jenkinson 1978; Li et al. 2004; Ge et al. 2010). Meanwhile, soil enzymes involved in the degradation of soil N are also important indicators of soil N transformation (Yang et al.

2008; Sardans et al. 2008; Nannipieri and Eldor 2009). Therefore, studying the links between these microbial properties and N transformation will enable us to better understand how soil underneath sealed areas is functioning; in particular, this will also provide a quantitative evaluation of the impact of soil sealing on soil quality and ecological functions (Lehmann and Stahr 2007; Zeng et al. 2009).

This study evaluated the soil inorganic N, net potential N-mineralization, nitrification rates, as well as the biotic and abiotic factors that drive these processes in three land-cover types of soils (impervious, forest and bare land), with an emphasis on the effects of soil sealing. By comparing soil properties and key process with nature forest and bare land, it is easy to find the main factors that significantly changed after soil sealing. This would be helpful to evaluate the degree of soil degradation and identify the critical soil properties and functions that need to be improved (Doran and Parkin 1994; Brejda et al. 2000; Gil-Sotres et al. 2005). It is hoped that this finding can provide additional insights on target soil management and practice reasonable remediation approaches in order to mitigate the negative impacts of soil sealing.

Urban land cover type and environment have been altered in Beijing due to the rapid economic development and drastic increase of urban population. Studies have shown that more than 70% of the lands in Beijing are estimated as high or middle imperviousness with the imperviousness index being larger than 70% or between 50% and 70% within the fifth ring road (Li et al. 2008). In addition, China is the third global N deposition country, with an atmospheric N deposition between 5.1 and 25.6 $\text{Nha}^{-1} \text{year}^{-1}$. More importantly, the wet N deposition in the center of Beijing is extremely serious with approximately 30.6 $\text{Nha}^{-1} \text{year}^{-1}$ (Liu et al. 2006), which makes this area very suitable for our research.

2 Materials and methods

2.1 Description of the study areas

The study site is located at (latitude) 39°92′–40°33′N and 116°46′–116°85′E in Beijing, China (Fig. 1). This area belongs to the temperate zone with an average annual temperature of 12°C and rainfall of 640 mm (more than 80% precipitating from June to August). Soils in the studied area are defined as sandy loam according to the USDA texture classification system. However, due to urban expansion and human activities, most of the green areas have been replaced by impervious land, which occupied more than 70% of the whole area. Meanwhile, the natural forest vegetation was replaced by *Pinus tabulaeformis*, *Quercus acutissima*, *Populus tomentosa*, and bush-grass communities (Li et al. 2005).

Three major land cover types (natural forest, bare land and impervious land) which effectively represent the changes of

Fig. 1 Location of the study area and sampling sites

land cover types and the degradation process of urban soils in Beijing, China, were included in this study. According to historical records and field survey, we carefully selected the natural forests and bare lands with the characteristics (such as plant species, soil texture and environmental conditions) were similar to previous reports before soil sealing. Furthermore, the natural forest sites without human disturbance for more than 10 years were far from the built up area. Three abandoned plant-free lands that were reserved for constructions were chosen as bare land. The impervious sites were mainly the old roadbeds and residential areas within fifth rings of Beijing. The sealing of the impervious soils had a history of over 10 years.

2.2 Soil sampling

The soils were sampled from May to June 2010. Three sites each were selected for forest and bare land, and six sites with similar soil parent materials were selected as impervious land (see Fig. 1). At each site, three replicate plots (1 × 1 m) were selected for each land cover types. All plots were on a level topography, and have the same management history and vegetation types. In each plot, six soil cores (2.5 cm in diameter) were randomly selected from the soil layers with a depth of 0–10, 10–20, 20–30 and 30–40 cm,

respectively. In the impervious sites, we punched holes on the hard ground and removed the padding to make the sealed soil accessible for sampling. After careful removal of the litter layer, roots and stones, soil samples from the same layer of each plot were mixed and divided into two subsamples. One subsample was air-dried for the analysis of soil physicochemical properties, and the other was sieved freshly through a 2-mm mesh and stored in plastic bags at 4°C. Soil microbial biomass, enzyme activities and $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ concentrations were measured at the same time within 48 h. Potential N mineralization and nitrification rate were analyzed as soon as incubation was completed.

2.3 Soil physical and chemical analysis

For the samples collected from each layer, soil moisture content (SMC) was determined gravimetrically by drying the samples in an oven at 105°C for 24 h. Soil texture was determined using Laster particle analyzer (Malvern Company (UK), mastersizer-2000). Soil pH was measured in a 1:2.5 (soil/water ratio) mixture using a glass electrode. Soil organic matter (SOM) was determined by the $\text{K}_2\text{Cr}_2\text{O}_7$ titration method as described previously (Lu 1999). Total C and N were analyzed by an automated instrument (Element analyzer Vario

EL Germany) and alkali-hydro N was measured by alkali-hydro diffusion method (Lu 1999).

2.4 Analysis of soil N-mineralization/nitrification rate and inorganic N concentration

Soil inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) was extracted with 2 mol l^{-1} KCl for 1 h and the concentration was determined by an auto-analyzer (continuous, flow analyzer [CFA]). Soil potential net N-mineralization rate was measured by the differences of total inorganic N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) before and after the incubation. Similarly, potential net N-nitrification rate was determined by the differences between the initial and final $\text{NO}_3^-\text{-N}$ during the incubation. Briefly, a 25-g fresh soil sample was weighted out and the moisture was adjusted to water-holding capacity before being incubated at 25°C for 28 days. At the end of the incubation, the soil samples were extracted with $100 \text{ ml } 2 \text{ mol l}^{-1}$ KCl to determine the final inorganic N concentration.

2.5 Determination of soil microbial biomass C and N

Soil microbial biomass C and N (C_{mic} / N_{mic}) was determined using the chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987). Briefly, three replicates of sub-samples (fresh weight of each sample: 25 g) were fumigated with alcohol-free CHCl_3 for 24 h at 25°C . After removal of the fumigant, the soil samples were extracted with $100 \text{ ml } 0.5 \text{ mol l}^{-1}$ K_2SO_4 for 30 min and then filtrated through filters (Whatman 42). The non-fumigated soil samples were processed similarly for the determination of background level. The filtered samples were frozen at -20°C for the analysis of organic C and TN. The organic C concentration was measured by total organic carbon analyzer (Tekmar-Dohrmann Co., USA). Soil C_{mic} was calculated as the difference between fumigated and non-fumigated samples, and $K_{\text{EC}}=0.45$ (Vance et al. 1987) was used to indicate the extraction efficiency. TN content was analyzed using alkaline potassium persulfate digestion-UV spectrophotometric method (GB 11894-89). Soil N_{mic} was estimated as the difference between fumigated and non-fumigated samples, using a K_{EN} of 0.54 (Brookes et al. 1985).

2.6 Analysis of the enzyme activities

Enzyme activities were analyzed according to the method described by Guan (1986). Soil urease activity was determined by using urea as the substrate. Briefly, $10 \text{ ml } 10\%$ urea solution and 20 ml citrate buffer (pH 6.7) were added to 5 g (dry weight) of soil samples before being incubated at 37°C for 24 h. After incubation, the soil samples were shaken for 30 min and filtrated until color was developed. Optical density (OD) at

578 nm was measured for the extractions within 40 min. Urease activity was expressed by the amount (mg) of $\text{NH}_3\text{-N}$ generated by 1 g soil at 37°C for 24 h.

For determination of protease activity, 4 g of soil samples (dry weight) was incubated for 24 h at 30°C with $20 \text{ ml } 1\%$ casein solution (prepared by citrate buffer, pH=7.4) and 1 ml methylbenzene. After incubation, $2 \text{ ml } 0.05 \text{ mol l}^{-1}$ H_2SO_4 and $12 \text{ ml } 20\%$ Na_2SO_4 were used to precipitate the protein. The solutions of soil sample were centrifuged for 15 min, and OD at 500 nm was measured for the color development. Samples without addition of substrate were used as background. Finally, protease activity was expressed by the amount (mg) of $\text{NH}_2\text{-N}$ generated by 1 g soil within 24 h at 30°C .

Nitrate reductase activity was determined using phenol disulfonic acid luminosity method. One gram of soil (dry weight) was placed in 100 ml canister decompression triangle bottle with 20 mg CaCO_3 and 1 ml KNO_3 . After mixing, 1 ml glucose was added as hydrogen donor. The air was extracted from the mixture for 3 min. The triangle flask were slightly strayed and incubated at 30°C for 24 h. After incubation, 50 ml water and 1 ml saturated solution of potassium alum was added to the triangle flask, then 20 ml of the mixed liquid was moved to a porcelain dish to dry. To develop color, 10% NaOH was used to adjust the solution to alkaline after 1 ml phenol disulfonic acid and 15 ml water were added. OD was measured at 450 nm . Sterilized soil (180°C , 3 h) was used as cross reference. Nitrate reductase activity was expressed by the amount (mg) of $\text{NH}_2\text{-N}$ generated by 1 g soil at 30°C for 24 h.

2.7 Statistical analysis

SPSS 16.0 and DPS were used for statistical analysis. Means and standard errors for the replicates of each variable were calculated. Differences in chemical and microbial properties among land cover types were tested by one-way analysis of variance (ANOVA) separately for the considered soil layer. Because there was significant interaction between land cover type and soil depth, two-way ANOVA analysis was performed to assess the effect of each factor and of the interaction by comparing the group means of independent variable among land cover types at different soil depth. The least significant difference (LSD) analysis was used for post-hoc multiple comparisons if the data were homogeneous; otherwise, Tamhane analysis was performed. Canonical correlation analysis was then performed on the whole data set to further investigate the dependent relationship between N transformation, soil physicochemical properties and microbial properties. The linear functions of the canonical variables for N transformation (U), soil physicochemical properties (V) and microbial properties (W) were expressed as below: $U = a_1X_1 + a_2X_2 + \dots + a_pX_p$; $V =$

$b_1Y_1 + b_2Y_2 + \dots + b_qY_q$; $W = c_1Z_1 + c_2Z_2 + \dots + c_rZ_r$. In which $a_1, a_2, \dots, a_p$ ($p=5$), $b_1, b_2, \dots, b_q$ ($q=5$), $c_1, c_2, \dots, c_r$ ($r=5$) are the undetermined coefficients, and there were maximum correlation coefficients among U , V and W .

If necessary, data were transformed prior to ANOVA, canonical correlation analysis to satisfy the requirement of normal distribution of data. Statistical significance was determined at a level $P \leq 0.05$, unless otherwise noted.

3 Results

3.1 Soil physical and chemical properties

The physical and chemical parameters of different land cover types and soil depth are presented in Table 1. Soil nutrient status was seriously affected by sealing of soils and changed obviously with depths. Soil SOM values ranged between 4.84 and 19.20 g/kg, and TN values varied between 2.63% and 10.75% — with the lowest value found in impervious sites, particularly in 0–10 cm soil layer. However, the C/N ratio in impervious sites was significantly higher than that in the forest and bare land. In addition, the C/N ratio decreased as the depth of the soil layer increased. The highest soil pH was observed in the impervious sites at each depth (0–10, 10–20, 20–30, 30–40 cm), while SMC was lowest in impervious sites. Particle size distribution was not significantly different among land covers and depths, except for the silt content with the order of forest > bare land > impervious sites. Land cover type $\times$ depth interaction was not significant for soil pH, SMC, TN and SOM, but was significant for soil C/N ratio (see Table 1).

3.2 Soil microbial biomass C and N

Soil C_{mic} and N_{mic} were significantly different among the three land cover types and various soil depths (Figs. 2 and 3). The highest C_{mic} and N_{mic} were observed in forest land, while the lowest C_{mic} and N_{mic} were observed in the impervious sites. In 0–10 cm soil layer, C_{mic} and N_{mic} in impervious sites was 0.9 and 10.3 times lower than those in bare land, while 24.8 and 41.1 times lower than those in forest, respectively. Along the soil depth gradient, C_{mic} was decreased in forest and increased in bare land and impervious sites (see Fig. 2), while N_{mic} was decreased in forest and bare land and increased in the impervious sites (see Fig. 3).

3.3 Soil inorganic N and potential N mineralization

Table 2 displayed the results of variations of soil inorganic N and potential N mineralization/nitrification. There were

significant differences between forest and impervious sites in the values of extractable NO_3^- , alkali-hydro N and net potential N mineralization. Significant interactions between land cover types and depths were observed in soil net potential mineralization and alkali-hydro N (see Table 2). In each depth of soil layer, the order of net potential mineralization and net potential nitrification was impervious sites < bare land < forest. In impervious sites, net potential mineralization decreased as the soil depth increased. In contrast, net potential nitrification increased as the soil depth increased. Alkali-hydro N in forest (especially in the depth of 0–10 cm) was higher than that in impervious sites. However, soil NH_4^+ -N was highest in the impervious sites in each depth. NO_3^- -N was highest in impervious sites in 30–40 cm and it was also increased along the soil depth gradient.

3.4 Soil enzyme activity

Soil enzyme activities were affected by land cover types at different degrees. Soil urease activity ranged from 0.05 to 1.29 mg product g^{-1} soil 24 h (Fig. 4). The activity of this enzyme was significantly affected by land cover types and soil depths, and the interaction of the two factors. The lowest urease activity was found in the impervious sites, followed by bare land and forest. Similar to microbial biomass N, urease activity in bare land and forest was decreased with the increases in depth. In contrast, urease activity in the impervious sites increased with the increases in soil depth.

Soil protease activity ranged from 1.97 to 3.45 mg product g^{-1} soil 24 h, and it was not significantly different among different land cover types (Fig. 5). The activity of this enzyme in impervious sites was higher than that in other land cover types in each depth, especially in the 20–30 cm soil layer. In addition, protease activity was increased in forest and decreased in bare land along the depth gradient. However, there was no regular change pattern along soil depths in impervious sites.

Nitrate reductase activity ranged from 0.006 to 0.019 mg product g^{-1} soil 24 h, and was significantly affected by land cover types and soil depths (Fig. 6). In the soil layer with a depth of 0–10 cm, the activity of nitrate reductase had an order of bare land > forest > impervious land, but in 30–40 cm layer, the order was reversed. There was no significant variation in nitrate reductase activity among different depths in impervious land, which was different from the other land cover types.

3.5 Relationships between soil properties and N transformation

Though canonical correlation analysis, the relationships between N transformation (NT), soil physicochemistry properties (PC) and microbial characteristics (MC) were statistically analyzed, and one pair of canonical variables (CVs) was

Table 1 Physical and chemical characterization of soils in various depths of soils with different land covers in Beijing

Soil depth (cm)	Land cover	pH (H ₂ O)	SMC (%)	TN (%)	SOM (g kg ⁻¹)	C/N	Particle size distribution (%)			Texture classification
							Clay (<0.002 mm)	Silt (0.002–0.05 mm)	Sand (0.05–2 mm)	
0–10	Forest	7.81 (0.12)a	15.25 (0.51)a	10.75 (1.85)a	19.20 (2.91)a	17.19 (0.79)a	8.14a	41.21a	50.65a	Sandy Silt loam
	Bare land	8.04 (0.21)a	13.01 (0.11)b	8.61 (0.32)b	7.69 (0.74)b	25.92 (2.01)a	6.94a	34.60a	58.46a	Sandy Silt loam
	Impervious land	8.45 (0.35)b	10.01 (0.16)a	6.08 (0.12)c	6.68 (1.71)b	41.38 (4.11)b	7.12a	27.83b	65.08b	Sandy loam
10–20	Forest	7.84 (0.08)	15.50 (0.29)	8.34 (0.32)	16.71 (1.07)	13.87 (1.50)a	4.38	31.79	63.83	Sandy Silt loam
	Bare land	7.89 (0.23)	12.67 (0.06)	6.36 (0.22)	6.31 (0.57)	23.12 (2.66)b	2.91	35.04	62.05	Sandy Silt loam
	Impervious land	8.75 (1.09)	12.52 (0.52)	5.39 (0.76)	6.76 (1.42)	36.27 (3.22)c	6.07	24.07	69.86	Sandy loam
20–30	Forest	7.94 (0.12)	15.25 (0.39)	6.81 (0.61)	11.79 (1.69)	13.27 (0.97)a	5.21	36.64	58.15	Sandy Silt loam
	Bare land	8.07 (0.16)	14.33 (0.17)	4.84 (0.79)	4.84 (0.87)	16.76 (2.01)ab	9.63	31.64	58.73	Sandy Silt loam
	Impervious land	8.76 (1.32)	13.47 (0.74)	4.22 (0.68)	8.17 (1.31)	26.36 (3.71)b	12.19	25.85	61.96	Sandy loam
30–40	Forest	7.95 (0.09)	13.25 (0.35)	5.08 (0.56)	10.27 (2.02)	11.63 (0.67)a	4.89	34.54	60.57	Sandy Silt loam
	Bare land	8.20 (0.31)	13.67 (0.36)	2.92 (0.68)	5.01 (1.02)	16.79 (1.98)ab	2.95	30.45	66.60	Sandy Silt loam
	Impervious land	8.99 (0.11)	13.11 (1.21)	2.63 (0.48)	7.32 (1.11)	19.73 (1.91)b	0.57	23.76	75.67	Sandy loam
Two-way ANOVA results										
Land cover type	*	ns	**	**	**	ns	*	ns		
Depth		ns	ns	**	*	**	ns	ns	ns	
land cover type × depth	ns	ns	ns	ns	*	ns	ns	ns		
^a All sites (land cover type)	**	*	**	**	**	ns	**	ns		

Standard errors of the means are given in parentheses

SMC soil moisture content, TN total nitrogen, SOM soil organic matter, C/N soil C: N ratio, ns not significant

^a All sites (land cover type) analyzed together with a one-way ANOVA: * $P<0.05$, ** $P<0.01$

Different letters in the same column indicate significant effects (at $P=0.05$ level) of land cover changes in a given soil layer. When there was no significant land cover × depth interaction, the mean separation applies to the mean of all four soil layers

Fig. 2 Changes of microbial biomass C in different land covers and soil depths in Beijing (error bars represent standard error). *X*-axis represents the different types of land covers; *Y*-axis represents the microbial biomass C. Different letters on the bars indicate significant difference between soil depths at $P \leq 0.05$

extracted for each group (Table 3). The CVs of the three groups are shown below:

1. Soil N transformation and physicochemical properties

$$U_1 = -0.987X_1 - 0.017X_2 - 0.055X_3 - 0.059X_4 + 0.034X_5$$

$$V_1 = 0.092Y_1 + 0.018Y_2 - 0.355Y_3 - 0.630Y_4 + 0.108Y_5$$

2. Soil N transformation and microbial properties

$$U_1 = -1.040X_1 + 0.240X_2 + 0.001X_3 - 0.109X_4 - 0.078X_5$$

$$W_1 = -0.614Z_1 - 0.339Z_2 - 0.175Z_3 + 0.038Z_4 - 0.038Z_5$$

Fig. 3 Changes of microbial biomass N in different land covers and soil depths in Beijing (error bars represent standard error). *X*-axis represents the different types of land covers; *Y*-axis represents the microbial biomass N. Different letters on the bars indicate significant difference between soil depths at $P \leq 0.05$

3. Soil physicochemical properties and microbial properties

$$V_1 = -0.160Y_1 - 0.029Y_2 + 0.389Y_3 + 0.550Y_4 - 0.123Y_5$$

$$W_1 = 0.652Z_1 + 0.335Z_2 + 0.164Z_3 - 0.178Z_4 - 0.108Z_5$$

The results showed that the key factors in NT, PC and MC were alkali-hydro N (X_1), TN (Y_4) and SOM (X_3), and C_{mic}/N_{mic} (Z_1/Z_2). The functions of CVs also indicated that TN, SOM, C_{mic}/N_{mic} , the activities of urease and nitrate reductase, were positively correlated with N mineralization and nitrification. However, pH, SMC, C/N ratio, protease had negative effects on net potential N mineralization, while positive effects on net potential N nitrification.

The first canonical correlations between the three groups (NT and PC, NT and MC, PC and MC) were highly significant ($r=0.911$, $r=0.888$, $r=0.851$, respectively) with a good fit of $P<0.01$, indicating a strong association among them. The importance of a given index was expressed by the value of the correlation coefficient for the canonical variable (Table 4). Trends within the first canonical variable for NT were much stronger for alkali-hydro N ($r=-0.987$) than others, and the canonical variable of PC and MC were also strongly correlated ($r=-0.909$ and -0.844) with alkali-hydro N. The high correlation ($r=-0.866$ and -0.734) between TN, SOM and the first canonical variable for NT further supported the importance of TN and SOM in the association between NT and PC. In addition, data from the canonical correlation coefficient between C_{mic}/N_{mic} and canonical variable of NT showed that C_{mic}/N_{mic} were significantly correlated with NT ($r=-0.828$ and -0.742). There was also a significantly correlation between C_{mic}/N_{mic} and the first canonical variable for PC ($r=0.776$ and 0.702). Further redundancy analysis revealed that approximately 20.6% of total variance in NT was explained by the first canonical variable of PC and 17.8% by the first canonical variable of MC.

4 Discussion

4.1 Variations of soil nutrient status at different types and depth

Changes of soil TN, SOM, C/N ratio and inorganic N are important indicators of the variation in soil nutrient status and soil quality. In this study, we showed that SOM was significantly correlated with TN, C/N ratio, urease, N_{mic} , alkali-hydro N and net potential mineralization, suggesting that SOM is an important indicator of the variation of soil quality and soil nutrient status caused by sealing of soils (see Table 3). Soil SOM and TN (in 0–10, 10–20, 20–30 and 30–

Table 2 Inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$), alkali-hydro N concentration and potential net N-mineralization/nitrification rates in various depths soils with different land covers in Beijing

Soil depth (cm)	Land cover	Extractable NH_4^+ ($\mu\text{g N g}^{-1}$)	Extractable NO_3^- ($\mu\text{g N g}^{-1}$)	Alkali-hydro N (ppm)	Net potential N mineralization (ppm)	Net potential N nitrification (ppm)
0–10	Forest	1.07 (0.08)a	2.17 (0.28)a	92.11 (4.53)a	7.78 (0.59)a	1.98 (0.46)a
	Bare land	0.90 (0.09)a	3.30 (0.50)b	26.85 (0.92)ab	2.56 (0.14)b	0.71 (0.09)a
	Impervious land	2.07 (0.34)a	1.66 (0.22)a	16.72 (0.85)b	2.48 (0.81)b	0.33 (0.08)a
10–20	Forest	0.79 (0.02)	2.11 (0.71)	69.31 (9.86)a	6.59 (1.12)a	1.30 (0.11)
	Bare land	0.65 (0.14)	2.92 (0.34)	19.71 (0.14)b	3.24 (0.75)b	0.64 (0.08)
	Impervious land	1.32 (0.11)	2.27 (0.25)	23.73 (3.82)b	2.27 (0.41)c	0.45 (0.07)
20–30	Forest	0.83 (0.03)	1.55 (0.16)	53.12 (4.99)a	3.62 (0.11)a	0.79 (0.08)
	Bare land	0.86 (0.09)	3.88 (0.86)	20.44 (0.14)b	2.31 (0.18)b	0.62 (0.11)
	Impervious land	1.18 (0.21)	2.32 (0.54)	22.80 (1.41)b	1.96 (0.18)c	0.53 (0.01)
30–40	Forest	0.84 (0.05)	1.24 (0.09)	43.15 (4.21)a	2.46 (0.14)a	1.13 (0.12)
	Bare land	0.54 (0.07)	2.25 (0.99)	45.15 (1.48)a	2.36 (0.11)a	0.66 (0.03)
	Impervious land	1.09 (0.07)	2.38 (0.34)	17.75 (0.64)b	1.77 (0.38)b	0.64 (0.05)
Two-way ANOVA results						
Land cover type	ns	**	**	**	*	
Depth		ns	ns	**	**	ns
land cover type $\times$ depth	ns	ns	**	**	ns	
All Sites (land cover type) ^a	*	*	**	**	**	

Standard errors of the means are given in parentheses. Different letters in the same column indicate significant effects (at $P=0.05$ level) of land cover changes in a given soil layer. When there was no significant land cover $\times$ depth interaction, the mean separation applies to the mean of all four soil layers

ns not significant

^a All sites (land cover type) analyzed together with a one-way ANOVA: * $P<0.05$, ** $P<0.01$

40 cm soil layers) in impervious sites were lower than those in the forest and bare land, implying that sealing of soils hindered the supply of nutrient and thus caused the degradation of soil fertility. The highest C/N ratio in impervious sites also suggested that N was the restrictive nutrient in this land cover type (Templer et al. 2005). Compared to bare land, the higher concentration of SOM and TN in forest was

mainly due to the vegetation, especially the accumulation of plant litter at the top soil layer.

Alkali-hydro N and inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$) are the characteristic N source available for microbe and plants, which could reflect the supply of N in soils in the near future (White and McDonnell 1988; Lorenz and Lal 2009). Sealing of soils could affect the soil physicochemical and microbial

Fig. 4 Urease activity in different land covers and soil depths in Beijing (error bars represent standard error). X-axis represents the different types of land covers; Y-axis represents the urease activity. Different letters on the bars indicate significant difference between soil depths at $P\leq 0.05$

Fig. 5 Protease activity in different land covers and soil depths in Beijing (error bars represent standard error). X-axis represented the different types of land covers; Y-axis represented the protease activity. Different letters on the bars indicate significant difference between soil depths at $P\leq 0.05$

Fig. 6 Nitrate reductase activity in different land covers and soil depths in Beijing (error bars represent standard error). X-axis represents the different types of land covers; Y-axis represents the nitrate reductase activity. Different letters on the bars indicated significant difference between soil depths at $P \leq 0.05$

properties, leading to the alteration of soil N availability. Consistent with many other reports (Badiane et al. 2001), our results showed that land cover change and the removal of vegetation significantly affected soil alkali-hydro N content. Correlation analysis suggested that the significantly decreased alkali-hydro N in impervious sites was correlated with the changes of SOM, C_{mic} , N_{mic} , urease and protease. The positive correlation between NH_4^+ -N and C/N ratio indicated that nutrient deficiency in the sealed soil limited the growth of microbes and stimulated microbes to decompose any available N into NH_4^+ -N for their survival. Meanwhile, the anaerobic conditions under impervious surface promoted the conversion from NO_3^- -N to NH_4^+ -N by nitrate reductase enzyme. According to Raghubanshi et al. (1990), NH_4^+ -N was the nutrient strongly demanded by plants during the growing season (May to June), and this

might be one of the explanations why the concentration of NH_4^+ -N was significantly decreased in forest, particular in the 10–20 cm soil layer. In addition, the concentration of NO_3^- -N in the impervious sites was positively affected by alkali-hydro N (see Table 3). Therefore, lack of plant litter inputs and imbalance between inputs and outputs of nutrients in the sealed soils may be the most important drivers to alter soil nutrient and functions (Zeng et al. 2009).

4.2 Effect of soil sealing on microbial biomass and enzyme activity

Soil sealing partially or completely prevents the exchange of gases, water and nutrients, and greatly altered soil physicochemical properties and nutrient availability, which ultimately affected soil microbial biomass and enzyme activities. Microbial biomass has been proposed as a sensitive indicator of soil quality and N flux due to its critical functions in soils and active roles in N transformation (Chang et al. 2007). Significant decreases of microbial biomass in impervious sites (see Fig. 2) reflected the great disturbance caused by sealing of soils. Reduced availability of carbon and substrates may be the key reason for the decreases in microbial biomass (Nannipieri and Eldor 2009). In addition, moisture stress, alkaline environment and poor ventilation caused by sealing of soils may all contribute to the reduction of microbial biomass (Tripathi and Singh 2009). However, destruction of vegetation and the alteration of soil nutrient status after soil sealing are the main factors affecting the synthesis and activities of soil enzymes (Chakrabarti et al. 2000).

Urease synthesis is regulated by soil N and is repressed when the preferred N source, e.g., NH_4^+ , was available (Mobley et al. 1995). In this study, we showed that impervious

Table 3 Wilk's lambda and chi-square test of canonical correlation coefficients

Item	Canonical vector no.	Canonical correlations	Wilk's lambda	χ^2	df	P
Soil N transformation and microbial properties	1	0.888	0.132	51.567	25	0.001**
	2	0.554	0.624	12.035	16	0.742
	3	0.257	0.901	2.693	10	0.975
	4	0.188	0.964	0.946	4	0.918
	5	0.032	0.999	0.026	1	0.872
Soil N transformation and physicochemical properties	1	0.911	0.099	58.928	27	0.001**
	2	0.522	0.581	13.832	17	0.611
	3	0.396	0.798	5.710	9	0.769
	4	0.219	0.948	1.366	4	0.850
	5	0.068	0.995	0.118	1	0.732
Soil microbial properties and physicochemical properties	1	0.851	0.171	45.037	25	0.008**
	2	0.494	0.619	12.222	16	0.729
	3	0.380	0.819	5.076	9	0.828
	4	0.185	0.958	1.094	4	0.895
	5	0.091	0.992	0.211	1	0.646

Wilk's, χ^2 and df are the values of Shapiro–Wilk's test, chi-square test and the freedom, respectively

* $P < 0.05$, ** $P < 0.01$

Table 4 Canonical correlation between N transformation and soil properties

N transformation	Correlation coefficient between N transformation and canonical variable of soil physicochemical properties	Correlation coefficient between N transformation and canonical variable of soil microbial properties
Alkali-hydro N (X_1)	−0.909	−0.844
Extractable NO_3^- (X_2)	−0.423	−0.171
Extractable NH_4^+ (X_3)	−0.015	−0.001
Net potential N mineralization (X_4)	−0.138	−0.261
Net potential N nitrification (X_5)	−0.083	−0.286
Soil physicochemical properties	Correlation coefficient between soil physicochemical properties and canonical variable of N transformation	Correlation coefficient between soil physicochemical properties and canonical variable of microbial properties
pH (Y_1)	0.501	−0.517
SMC (Y_2)	−0.145	0.105
SOM (Y_3)	−0.734	0.690
TN (Y_4)	−0.866	0.790
C/N (Y_5)	0.563	−0.551
Soil microbial properties	Correlation coefficient between soil microbial properties and canonical variable of N transformation	Correlation coefficient between soil microbial properties and canonical variable of soil physicochemical properties
MBC (Z_1)	−0.828	0.776
MBN (Z_2)	−0.742	0.702
Urease (Z_3)	−0.574	0.538
Protease (Z_4)	0.231	−0.339
Nitrate reductase (Z_5)	−0.487	0.356

The proportions of variance of N transformation, soil physicochemical and microbial properties explained by their own and opposite canonical variables were calculated by Redundancy Analysis

sites had the lowest soil urease activity compared to bare land and forest, which was possibly due to the increasing level of NH_4^+ in the sealed soil layers. Moreover, we also showed that urease activity in the impervious sites was affected by SOM, TN, C_{mic} , N_{mic} , alkali-hydro N, pH and C/N ratio, which was consistent with the report of Reynolds et al. (1985). In contrast, the impervious sites had the highest protease activity compared to bare land and forest, implying that protease synthesis was not depressed by sealing of soils, and the protein or chitin substrates was not limited in soils. Inconsistent with the studies of Geisseler and Horwath (2008), we showed in this study that the protease activity was negatively correlated with SOM, N_{mic} and alkali-hydro N, but was not significantly influenced by NH_4^+ -N level in the soils. The activity of nitrate reductase was regulated by NO_3^- -N concentration, and it contributed to the synthesis of NH_4^+ -N in impervious lands. However, significant correlation among them was not observed.

4.3 Effects of soil sealing on N transformation

Soil net potential mineralization and nitrification rate in impervious sites was significantly decreased, indicating that

N transformation was influenced by sealing of soils. Soil microbes play a very important role in the process of N mineralization and nitrification. Sealing of soils cut off the nutrient supply and caused the reduction of SOM and TN. Changes of soil physicochemical properties, e.g., pH, SMC and particle size, after soil sealing affect the environment for soil microbial growth. Lack of nutrients and alteration of the environment reduces microbial biomass, which results in the reduction of mineralization. Variation in soil enzyme activities affected by soil properties and microbial functions also causes the reduction of N transformation in impervious sites (Reynolds et al. 1985; Geisseler et al. 2010). Moreover, sealing of soils prevents the exchanges of water and gas and results in the formation of a dry and oxygen-poor soil environment, which ultimately inhibits the growth of nitrifying bacteria and influences N nitrification. However, soil microbial biomass, TN and SOM were the key factors that affected soil N transformation after sealing of soils, while the changes of soil pH, C/N ratio and urease, especially in 0–10 cm soil depth, also contributed to N transformation. In this study, the accumulation of NH_4^+ -N and the increasing level of NO_3^- -N in sub-soils were observed in impervious sites, suggesting that sealing of soils influences the

distribution of inorganic N and aggravate the potential pollutions of urban groundwater, rivers and lakes.

5 Conclusions and recommendations

In summary, there was a significant difference in microbial biomass, enzyme activity and N transformation among forest, bare land and impervious sites, especially in the top 10-cm soil layer. Net potential N mineralization and nitrification were decreased in impervious sites due to the alteration of soil physicochemical properties and microbial characteristics. In contrast to nature sealing, artificial soil sealing in urban areas was generally extensive and permanent, and entails a modification of the neighboring unsealed areas. Therefore, we suggested that reducing the negative effects of soil sealing could be useful measures to improve the function of urban soils (such as storage, regulating, filtering and transformation; biomass production and biological habitat) and hence facilitate the remediation of adverse impacts caused by urban N deposition. In the paved areas, permeable or semi-pervious pavement systems would be helpful to limit the consequences of soil sealing. The highly conductive materials and the seams among the pavements could allow minimal exchange of gas, water and solute between soils under sealing and their environment (Scalenghe and Marsan 2009). In the unsealed areas, the application of mulch and rock fragment cover was a good approach to protect soil from sealing by raindrop impact (Kochenderfer and Helvey 1987). Setting low elevation greenbelt adjacent to the sealed area was also a useful measure to increase the water infiltration and to reduce the runoff of rainstorm caused by soil sealing.

Although the findings in this study have provided some insights into the influence of soil sealing on N transformation and microbial properties in urban soils, there are still several limitations. Given the difficulties in selecting appropriate experimental sites and in sampling soils under impervious surfaces in the metropolis, we only selected quite a low number of study sites in this study. Therefore, subsequent research should focus on evaluating the effects of different sealing types, ages and the scales of impervious pavement on urban soil microorganism and nutrient availability. In addition, the factors including soil temperature and heavy metal should also be considered to better understand the underlying mechanisms.

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