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Subtropical urban turfs: Carbon and nitrogen pools and the role of enzyme activity

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ABSTRACT

Urban grasslands not only provide a recreational venue for urban residents, but also sequester organic carbon in vegetation and soils through photosynthesis, and release carbon dioxide through respiration, which largely contribute to carbon storage and fluxes at regional and global scales. We investigated organic carbon and nitrogen pools in subtropical turfs and found that dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were regulated by several factors including microbial activity which is indicated by soil enzymatic activity. We observed a vertical variation and different temporal patterns in both soil DOC, DON and enzyme activities, which decreased significantly with increasing soil depths. We further found that concentration of soil DON was linked with turf age. There were correlations between grass biomass and soil properties, and soil enzyme activities. In particular, soil bulk density was significantly correlated with soil moisture and soil organic carbon (SOC). In addition, DOC correlated significantly with DON. Significant negative correlations were also observed between soil total dissolved nitrogen (TDN) and grass biomass of *Axonopus compressus* and *Zoysia matrella*. Specifically, grass biomass was significantly correlated with the soil activity of urease and β-glucosidase. Soil NO_3-N concentration also showed negative correlations with the activity of both β-glucosidase and protease but there were no significant correlations between cellulase and soil properties or grass biomass. Our study demonstrated a relationship between soil C and N dynamics and soil enzymes that could be modulated to enhance SOC pools through management and maintenance practices.

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Introduction

Urban ecosystems are exposed to higher air and soil temperatures than their surroundings due to urban heat island (UHI) effect, and thus are more vulnerable to global warming. One of the consequences of the UHI effect is an increase in respiration rates in urban vegetation and soils due to elevated temperature, which in turn leads to enhanced microbial and soil enzyme activities (Davidson and Janssens, 2006; Karhu et al., 2014). Allison et al. (2010) presented a microbial-enzyme model in which microbial biomass and soil enzymes work together as catalysts in the conversion of soil organic carbon (SOC) to dissolved organic carbon (DOC), which is key step for SOC decomposition. In addition, SOC accounts for more than 60% of the global soil C pool, which is more than three times the size of the

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atmospheric pool and plays a critical role in the soil C fluxes and global C cycle (Lal, 2004).

Dissolved organic matter (DOM) plays a critical role in the ecosystems as it mediates many chemical and biological reactions (Chantigny, 2003). DOC refers to all dissolved organic C-containing molecules in water or soils (McDowell and Likens, 1988). Despite the fact that DOC accounts for only a small part of the soil C, it is involved in many important soil biological processes and serves as a transfer means for C between ecosystems, regulating the sequestration and export of SOC pools. On the other hand, urban ecosystems have lower C inputs than natural ecosystems due to less litter addition, and consequently lower decomposition rate and C release (Churkina, 2012). Therefore, it represents a critical factor in global C cycle.

In addition to DOC, dissolved organic nitrogen (DON) is another major component of the soil microenvironment. DON commonly refers to the organic forms of nitrogen such as polypeptides, free amino acids or other nitrogenous organic materials either excreted by living organisms or released from decomposed life forms (Neff and Asner, 2001; Qualls and Richardson, 2003). In soils, DON is present in two different pools: amino acids and proteins which were readily decomposed by microbes; and high molecular weight DONs that undergo slow turnover (Jones et al., 2004). In ecosystems with little anthropogenic N input, vegetation and hydrology play critical roles in N retention and loss (Cairns and Lajtha, 2005). In general, DON is a major source of N for microbes and plants, which in turn contribute to local climate changes by generating as well as absorbing various greenhouse gases. Thus, DON contributes both directly and indirectly to local and global ecosystems. Interactions among microbes, plants and soil control the level of DON and DOC, and affect climate change.

Soil enzymes play a key role in nutrient cycling which involves many biochemical processes in terms of degradation, transformation and mineralization of soil organic materials (Dick et al., 1996; Sinsabaugh, 2010), thereby contributing to the stock and export of soil DOC and DON pools. On the other hand, soil enzymes have been reported to be sensitive to soil conditions such as moisture, temperature and field management practices (Bandick and Dick, 1999; Green and Oleksyszyn, 2002) such as tillage (Kandeler et al., 1999b), burning and fertilization (Ajwa et al., 1999).

However, little is known about the role of soil enzymes in regulating the stock and export of soil organic matter in urban ecosystems. Therefore, SOC, DOC, DON, soil

Table 1 – Turf sites studied in Hong Kong and Shenzhen, China.

Turf sites	Year of establishment	No. of points sampled	Turf size (m^2)	Turfgrass species
Hong Kong				
HK-01	1957	15	20000	*A. compressus*
HK-02	1963	9	7140	*Z. japonica*
HK-03	1963	9	7140	*A. compressus*
HK-04	1968	9	2800	*A. compressus*
HK-05	1968	9	6000	*A. compressus*
HK-06	1968	9	1700	*A. compressus*
HK-07	1970	18	4928	*A. compressus*
HK-08	1988	18	2500	*A. compressus*
				Z. japonica
HK-09	1991	18	20000	*A. compressus*
HK-10	1997	18	10130	*A. compressus*
HK-11	1998	15	1800	*A. compressus*
				Z. matrella
HK-12	2010	15	9000	*C. dactylon* × *C. transvaalensis*
				Z. matrella, *L. perenne*
HK-13	2010	9	1020	*A. compressus*
HK-14	2010	9	2000	*Z. japonica*
Shenzhen				
SZ-01	1961	9	4000 *	*Z. matrella*
SZ-02	1982	9	20000 *	*A. compressus*
SZ-03	1984	9	5000 *	*Z. matrella*
SZ-04	1988	9	10000	*A. compressus*
SZ-05	1997	15	160000	*A. compressus*
				Z. matrella
SZ-06	1998	9	2000 *	*Z. matrella*
SZ-07	1998	9	60000	*Z. matrella*
SZ-08	1999	15	60000	*Z. matrella*
SZ-09	1999	15	400000	*A. compressus*
				Z. japonica
SZ-10	2004	9	10000 *	*Z. matrella*
SZ-11	2006	9	10000 *	*Z. matrella*
SZ-12	2007	9	60000	*Z. matrella*
SZ-13	2011	9	10000 *	*Z. matrella*
SZ-14	2011	15	120000 *	*Z. matrella*

* Turf size estimated from aerial photos.

respiration, microbial biomass and enzyme pools should be investigated further in urban ecosystems to determine their interactive influences on C cycle at multiple scales in local, regional and global levels. This study assessed (1) DOC and DON exports and their effects on soil C pool in urban turfs; and (2) soil enzyme activities and their role in governing soil DOC and DON pools in urban turfs with different grass species in metropolitan Shenzhen and Hong Kong in southern China.

1. Materials and methods

1.1. Site description

We studied selected urban turfs in Hong Kong (22°15′44″N, 114°10′ 41″E) and Shenzhen (22°32′43″N, 114°04′05″E), China. We chose turfs according to the following criteria: (1) turf area larger than 1000 m^2; (2) grass species commonly employed in subtropical cities in China; and (3) different types of turfs with wide turf ages, including lawns (located in urban parks and campus), athletic (football and cricket) fields, green roofs, roadside green belts, which were established from 1957 to 2011 (Table 1).

There were five grass species in the studied turfs. *Axonopus compressus* was found in most turfs in Hong Kong, while *Zoysia matrella* was the dominant species in Shenzhen. *Zoysia japonica*, *Cynodon dactylon* × *C. transvaalensis* and *Lolium perenne* were also grown in sports fields in Hong Kong. We collected soil and grass samples from 14 turfs in Hong Kong and another 14 in Shenzhen (Table 1) during the wet season from 15 August to 27 September 2012.

1.2. Soil sampling and analysis

The sampling followed the methods as described by Kong et al. (2014). We selected 9–18 points based on the size of turf, types of grass species and locations for soil sampling from all the field turfs. Generally, for turfs with one grass species, 9 points were sampled for park size below 10,000 m^2 and 15 points for size above 10,000 m^2. For turfs with more than two grass species, 15 points were sampled. Specifically, 18 points were sampled for turfs located far from each other in the same park, including Sha Tin Park (STP), Kowloon Park (KLP), Yuen Long Park (YLP) and Tai Po Waterfront Park (TPWP).

Soils were sampled from 0 to 5 cm, 5–10 cm and 10–15 cm with a soil corer of 5 cm in diameter and 20 cm in length. Samples were placed in plastic bags and delivered to the laboratory for analysis. Twenty grams of field-moist soils were extracted by 100 mL distilled water and filtered through Whatman No. 1 papers. Soil filtrates were used for the determination of ammonium-N (NH_4-N), nitrate-N (NO_3-N), DON and DOC concentrations. NH_4-N and NO_3-N were determined using a SAN++ Segmented Flow Analyzer (Skalar Analytical BV, Breda, Netherlands). Total dissolved nitrogen (TDN) was determined using a Shimadzu TOC 5000A Total Organic Carbon Analyzer with a TNM-1 total nitrogen detector. DON was calculated by the difference: DON = TDN – (NO_3 + NH_4)-N. DOC was determined by the TOC 5000A TOC Analyzer. Measurement of soil pH, soil water content and SOC followed the methods as described by Kong et al. (2014). We then performed Pearson Correlation Analysis between DOC and DON both in soil and grass shoot biomass, temperature, and soil water content for all locations.

To understand the contribution of soil enzymes to DOC and DON, we determined soil enzymes in similar fashion as described above. Specifically, we measured the enzyme activities of the soil samples collected from the urban turfs in Hong Kong and Shenzhen. Both vertical changes and age correlation were compared among all the turf sites.

1.3. Grass sampling and C analysis

Grass sampling and C content analysis followed the methods by Kong et al. (2014). Briefly, aboveground grass shoots (25 × 15 cm^2) were collected from all turfs, and oven dried at 105°C for 48 hr to obtain the dry-weight biomass. Grass samples were cut and analyzed with a TOC analyzer to determine the concentrations of TC, IC and OC, which were used to calculate the amount of grass C stock by multiplying TOC concentration (%) by the weight of the sample and expressed in g/m^2.

1.4. Soil enzyme activity analysis

1.4.1. Urease activity analysis

We analyzed enzyme activity using modified methods (Kong et al., 2009). Specifically, urease activity was determined by Nessler reagent spectrophotometric method (Klose and Tabatabai, 2000; Sinsabaugh et al., 2000). One gram soil sample was sieved (<1 mm) and placed in a 15 mL centrifuge tube, added with 2 mL phosphate buffer of pH 6.7 and 0.1 mL toluene, and mixed for 15 min. Two milliliter 10% urea solution (substrate) was added to the sample and incubated at 37°C for 48 hr. After incubation, 4 mL of 1 mol/L KCl were added to the sample, shaken thoroughly and then centrifuged at 4000 r/min (Versatile Refrigerated Centrifuge, Techcomp, CT15RT) for 10 min and filtered. One milliliter of the filtrate was added to 25 mL of distilled water, then 1 mL of potassium sodium tartrate and 0.8 mL of Nessler Reagent (compounds of HgI_2 and KI) were added, shaken gently, after which 4 mL NaOH solution added carefully, and diluted to 50 mL with distilled water. The released NH_4-N was determined at 460 nm using UV-2000 spectrophotometer. Urease activity was expressed as μg NH_4-N/(g soil·hr).

1.4.2. β-glucosidase activity analysis

β-glucosidase activity was determined colorimetrically for p-nitrophenol released by β-glucosidase (Dick et al., 1996; Knight and Dick, 2004). One gram of soil was placed in a 15 mL centrifuge tube, 0.9 mL distilled water and 0.1 mL toluene were added to the sample, and then mixed thoroughly for 15 min. Then 1.5 mL acetate buffer (pH 4.8) and 0.6 mL 5×10^{-3} M p-nitrophenol-β-D-glucopyranoside solution were added to the sample, mixed thoroughly and incubated at 37°C for 1 hr. Seven milliliters of ethanol was added to the solution, then shaken thoroughly and centrifuged at 4000 r/min for 10 min and filtered. β-glucosidase activity was determined at 400 nm in a spectrophotometer, and was expressed as μg p-nitrophenol/(g soil·hr).

1.4.3. *Cellulase activity analysis*

Cellulase activity was measured in a phosphatase buffer (pH 5.5) with 1% carboxymethyl cellulose (CMC) as the substrate (Semenov et al., 1996; Criquet, 2002; Doyle et al., 2006). One gram of sieved (<1 mm) soil was placed in a 15 mL centrifuge tube, and 5 mL pH 5.5 phosphate buffer and 0.5 mL toluene were added, and mixed thoroughly for 15 min. One milliliter of 1% cellulose solution (substrate) was added, sealed and incubated at 37°C for 48 hr. At the end of incubation, the mixture was heated up to 100°C to terminate the reaction and then cooled under running tap water. Potassium alum (0.3 g) was added to the mixture to make undissolved CMC flocculate and settle. The mixture was then centrifuged at 4000 r/min for 10 min, and filtered. The filtrate (0.5 mL) was diluted with 2.0 mL distilled water, and 5 mL anthrone added carefully within ice water bath, cooled down, sealed with the cap, shaken gently and placed in boiling water bath for 10 min. The absorbance of reducing sugars was measured by a spectrophotometer at wavelength 540 nm. The cellulase activity was expressed as μg glucose/(g soil·hr).

1.4.4. *Protease activity analysis*

Soil protease activity was determined using Folin–Ciocalteu reagent by measuring the tyrosine released (Kandeler et al., 1999b; Müller and Bordusa, 2000). One gram of soil sample was placed in a 15 mL centrifuge tube, to which 1 mL pH 8.0 Tris–HCl buffer and 0.5 mL toluene were added and mixed for 15 min. Then 2 mL 1% casein solution were added, shaken briefly, and incubated at 37°C for 24 hr. At the end of incubation, 3 mL of 15% trichloroacetic acid were added, and the sample was shaken thoroughly and centrifuged at 4000 r/min for 10 min, then filtered. One milliliter filtrate was added with 5 mL 0.4 mol/L Na_2CO_3 and 1 mL Folin–Ciocalteu reagent, shaken thoroughly, and placed in an incubator at 37°C for 15 min for color development. The absorbance of the tyrosine released from the reaction was determined at 680 nm using a spectrophotometer. The protease activity was expressed as μg tyrosine/(g soil·hr).

1.5. Statistical analysis

All statistical analyses were done with SPSS statistical software (IBM SPSS 20.0, IBM Corporation). One-way ANOVA with Tukey's honestly significant difference (HSD) test was performed for nutrient contents and soil enzyme activities at different soil depths. Linear regression analysis was employed for soil C and N, and soil enzyme activity against turf age. Pearson's correlation analysis was performed between soil physical and chemical properties, soil enzyme activity and grass biomass. Principal component analysis (PCA) was employed for soil physical and chemical properties, grass biomass carbon density, and soil enzyme activities at depth 0–5 cm and 5–10 cm in the studied turfs.

2. Results

2.1. Physical and chemical properties of soils

Most of the soil samples had pH values <7.0, with exceptions for turf HK-10, HK-04, SZ-05, SZ-09 and SZ-06 (Table 2). Soil

Table 2 – Grass C density, soil properties and soil C content of studied turfs.

Turf sites	Bulk density (g/cm^3)	Water content (%)	pH	Grass C density (g/m^2)	SOC concentration (%)		
					0–5 cm	5–10 cm	10–15 cm
HK-01	1.02	18.7	6.3	166	0.36	0.00	0.00
HK-02	1.44	17.8	6.6	117	0.56	0.07	0.00
HK-03	1.46	12.9	6.7	159	1.86	0.56	0.00
HK-04	1.18	19.3	7.1	135	1.84	1.22	0.63
HK-05	1.26	16.8	6.6	69.8	1.53	0.89	0.30
HK-06	1.06	21.8	6.2	103	1.64	1.04	0.76
HK-07	1.24	24.0	6.5	120	2.55	1.04	0.59
HK-08	1.12	22.9	6.5	88.0	1.66	1.21	0.17
HK-09	1.07	19.5	6.0	133	1.24	0.71	0.24
HK-10	1.19	21.3	7.2	81.2	0.89	0.33	0.00
HK-11	0.97	23.2	6.0	96.2	3.76	1.13	0.91
HK-12	1.48	15.9	6.9	48.4	2.77	0.88	0.77
HK-13	1.46	18.7	6.5	159	2.99	2.25	1.25
HK-14	1.11	25.4	6.5	203	1.16	0.54	0.44
SZ-01	1.12	19.7	6.9	202	2.91	1.66	0.08
SZ-02	0.98	24.6	6.9	208	3.99	1.14	0.60
SZ-03	1.12	26.2	6.5	191	2.15	0.45	0.65
SZ-04	0.98	27.0	6.7	185	0.91	0.99	0.23
SZ-05	0.89	21.1	7.2	209	1.24	0.39	0.21
SZ-06	1.26	15.6	7.0	283	2.08	0.00	0.00
SZ-07	1.16	15.3	6.7	204	3.29	1.77	1.77
SZ-08	1.13	11.2	6.7	107	0.28	0.00	0.00
SZ-09	1.32	15.5	6.5	122	1.99	0.67	0.00
SZ-10	1.34	9.40	7.4	213	4.38	1.52	0.55
SZ-11	1.06	31.8	6.9	291	0.55	0.19	0.21
SZ-12	1.18	18.1	6.9	220	2.90	0.79	0.00
SZ-13	1.31	15.8	6.8	152	0.02	0.04	0.00
SZ-14	0.98	12.8	6.9	221	0.88	0.08	0.21

Table 3 – Results of two-way ANOVA of aboveground biomass of grass between *A. compressus* and *Z. matrella*, Hong Kong and Shenzhen.

Source	SS	df	MS	F value	p value
Grass	89,546.2	1	89,546.2	10.041	0.004 **
City	53,669.0	1	53,669.0	6.018	0.022 *
Grass × City	7589.2	1	7589.24	0. 851	0.366
Error	205,112	23	8917.95		
Corrected total	561,910	26			

ANOVA: analysis of variance
* Denotes significance at $p < 0.05$
** Denote significance at $p < 0.01$.

bulk density ranged between 0.89 and 1.48 g/cm^3. Soil water contents varied from 9.4% to 31.8% in the studied turfs.

2.2. C stock in turfgrasses

The C concentrations of turfgrasses ranged from 41.9% to 45.9%, which were used for C density calculation. The aboveground biomass (AGB) and C density of turfgrass in the studied turfs in Hong Kong and Shenzhen are shown in Table 2. Turfgrass biomass ranged from 121 g/m^2 in turf Hong Kong Cricket Club (HK-12) with *Cynodon dactylon* × *C. transvaalensis* to 728 g/m^2 in turf Shen Nan Roadside (SZ-11) with *Z. matrella*. Accordingly, C density of turfgrass varied from 48.4 in HK-12 to 291 g/m^2 in SZ-11 (Table 2).

We further compared the biomass of the two dominant species *A. compressus* and *Z. matrella* between Hong Kong and Shenzhen, which suggests that biomass of these two grasses differed significantly between species ($p < 0.05$) (Table 3).

2.3. Vertical variation in soil C

Soil total carbon (STC) concentration equals to SOC in the absence of inorganic carbon (IC) in our soil samples with pH ≤ 7.0. SOC in samples collected in three soil layers are shown in Table 2. In general, SOC concentrations decreased with soil depth, with the highest value in the surface 5 cm, followed by 5–10 cm, with the lowest in 10–15 cm. Analysis of variance (ANOVA) for SOC concentration between different soil depths revealed that significant difference ($p < 0.01$) was observed between top (0–5 cm) and middle layers (5–10 cm), and between middle and lower layers (10–15 cm). For 0–5 cm soil, SOC concentrations varied in the turfs, among which GEP showed the highest value of 4.38% (Table 2). Similarly, SOC density varied among the turfs and decreased with soil depth (Fig. 1), with significantly higher values at the surface 5 cm ($p < 0.05$) than layers below, and significant difference between middle and down layer ($p < 0.05$). Total SOC density at the top 15 cm soil was the highest with 4.88 kg C/m^2 in HK-13, while the lowest was 0.04 kg C/m^2 in SZ-08 (Appendix A Fig. S1).

In line with SOC, DOC was the highest at the top 5 cm soil layer, with siginificantly higher values than the layers below, but with no significant differences between middle and lower layers (Fig. 1). Among them, HK-01, HK-09 and HK-07 had the highest of 9.12, 18.6 and 25.6 μg C/g soil respectively at the top 5 cm, with their lowest of 3.52, 7.24 and 14.9 μg C/g soil at 10–15 cm soil. HK-04, HK-05 and HK-06 had relatively low DOC concentrations ranging between 0.78 and 5.74 μg C/g soil, but 10–15 cm had higher DOC than the upper layers, which was 5.74, 1.25, and 1.94 respectively (Appendix A Fig. S1). Our results show that both SOC and DOC were depth-dependent across all turfs studied (Fig. 1).

2.4. Vertical variation in soil N

Similar to SOC, The concentrations of TDN, NH_4-N and NO_3-N in the grassed soil peaked at the top 5 cm soil, with significant higher values than the layers below ($p < 0.05$, Fig. 2). Specifically, for concentrations of TDN, significant difference was observed between top 5 cm soil than below layers while no significant difference between middle and down layer of soil samples. For concentrations of NH_4 at the top 5 cm soil were significantly higher ($p < 0.05$) than the middle layer. Concentration of NO_3 was significantly lower ($p < 0.05$) at 10–15 cm than the top layer (0–5 cm), which was also lower than middle layer ($p < 0.05$). Significant difference in NO_3 was also noticed between top and middle layer ($p < 0.05$). However, we did not observe any significant difference in concentrations of DON different layers of soil.

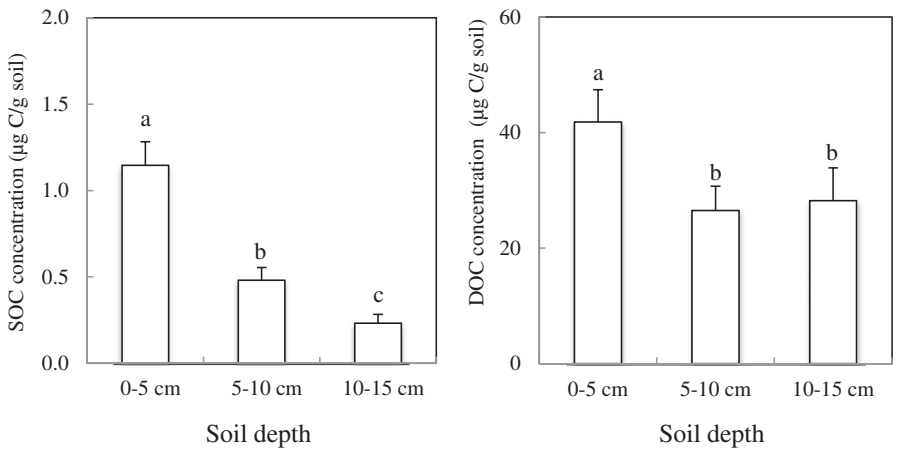


Fig. 1 – One-way ANOVA test for soil organic carbon (SOC) density and dissolved organic carbon (DOC) concentration at depths 0–5, 5–10 and 10–15 cm in the studied turfs ($N = 28$, error bars denote standard error of the mean (SEM); different letters denote significant difference at $p < 0.05$ according to Tukey's honestly significant difference (HSD) test).

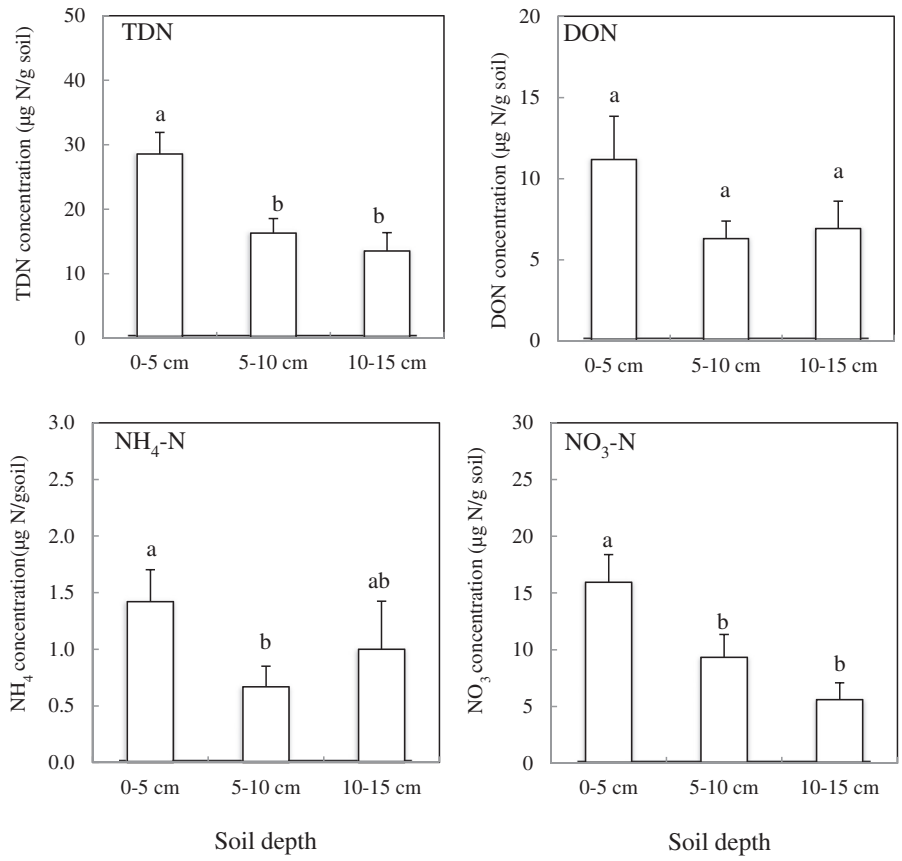


Fig. 2 – One-way ANOVA test for soil N density at depths 0–5, 5–10 and 10–15 cm in the studied turfs (TDN: total dissolved nitrogen; DON: dissolved organic nitrogen; NH_4-N: ammonia nitrogen; NO_3-N: nitrate-nitrogen; N = 28; error bars denote standard error of the mean (SEM); different letters denote significant difference at $p < 0.05$ according to Tukey's HSD test).

NO_3-N accounted for the major portion of soluble N in urban turfs. We found soil NH_4-N concentrations lower than 5.6 μg N/g at 0–5 cm, and 2.0 μg N/g soil at 5–10 cm, with one exception at 10–15 cm in HK-12 at 11.7 μg N/g soil (Appendix A Fig. S2). While higher NO_3-N concentrations were in all soil samples at the uppermost layer (Appendix A Fig. S2), with the peak value of 40.4 μg N/g soil in MP1, while MP3 and DHP had their highest concentrations at 10–15 cm. As DON = TDN –(NO_3-N + NH_4-N), the concentration of other N parameters in the studied turfs showed similar vertical pattern, with more turfs having higher DON at 10–15 cm soil (Appendix A Fig. S2).

2.5. *Vertical variations in soil enzyme activity*

Soil enzyme activity showed similar vertical variation, with significant higher values at the top 5 cm soil than layers below for β-glucosidase, urease and cellulase ($p < 0.05$), while no significant differences were observed for proteinase between different soil depths (Fig. 3). Specifically, β-glucosidase shown higher activity in the top 5 cm than the below 10–15 cm, while no significant difference was found between middle and other layers of soil. Urease and cellulase activities were observed with similar vertical pattern, with significantly higher value at the top 0–5 cm layer than the layers below only (Fig. 3).

Furthermore, urease activity was the highest at the top soil layer for most turfs, while the soil at 10–15 cm showed higher urease activity in HK-07, HK-09, SZ-04, HK-14, SZ-07, SZ-02 and SBSZ-14 (Appendix A Fig. S3). Among all turfs, LZP had the highest urease activity at 176 μg NH_4-N/(g soil·hr) at 10–15 cm, which may be due to its higher fertilizer inputs.

The surface 0–5 cm soil contained higher activity of β-glucosidase in most turfs, except HK-10, SZ-03 and SZ-01 where the β-glucosidase activity was highest at 10–15 cm. The peaked β-glucosidase activity was found in SZ-11 at 33.2 μg p-phenol/(g soil·hr) (Appendix A Fig. S3).

Similarly, the top 5 cm had the highest cellulase activity in most turfs, with the exceptions of HK04, HK-06, SZ-13, HK-03, HK-01 and SZ-07 (Appendix A Fig. S3). Among all turfs, SZ-03 had the highest cellulase activity at 0–5 cm of 127 μg glucose/(g soil·hr), and SBP the lowest at 8.6 μg glucose/(g soil·hr).

In contrast to other enzymes, protease showed little variations (Appendix A Fig. S3), ranging between 114 μg tyrosine/(g soil·hr) in HK-02 and 300 μg tyrosine/(g soil·hr) in ZSP at 0–15 cm. The difference between surface layer (0–5 cm) and 5–15 cm soil layers was not as obvious as the other three enzymes. HK-02, HK-09, HK-03, HK-01 and HK-14 had relatively lower protease activities than other turfs.

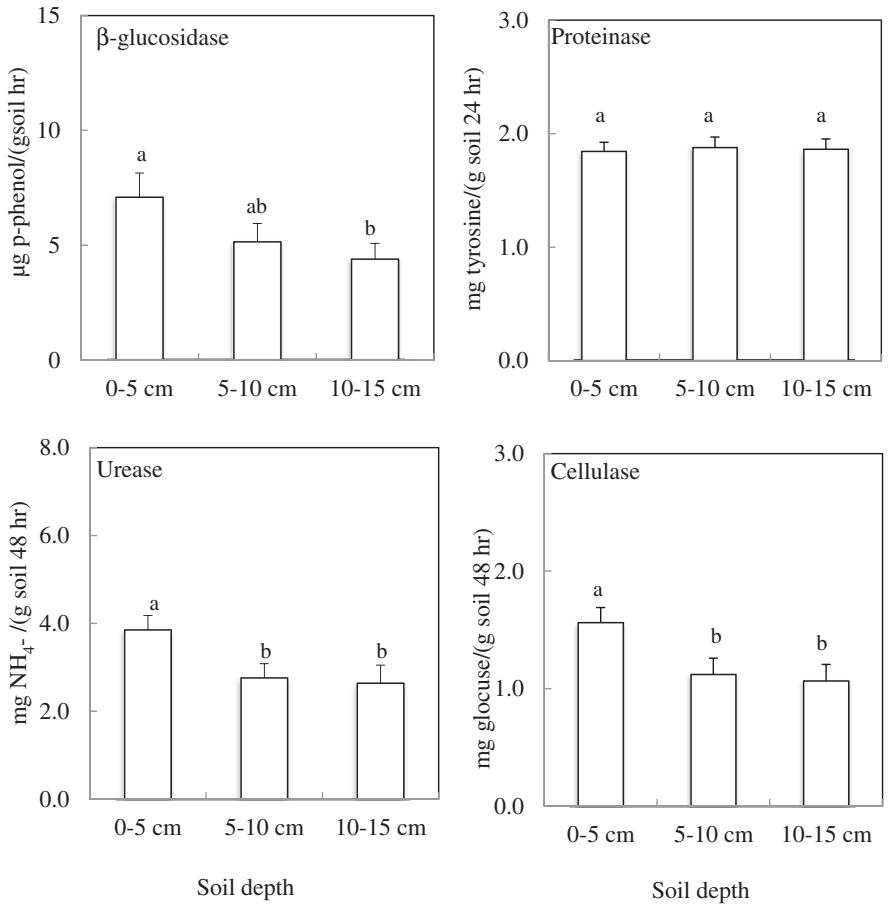


Fig. 3 – One-way ANOVA test for soil enzyme activities at depths 0–5, 5–10 and 10–15 cm in the studied turfs ($N = 28$; error bars denote standard error of the mean (SEM); different letters denote significant difference at $p < 0.05$ according to Tukey's HSD test).

2.6. Turf age correlation with soil DOC and DON, and soil enzyme activity

DOC concentrations in the soil samples from urban turfs did not show significant change with turf age (Fig. 4a). Contrastingly, we observed a weak correlation between DON and turf age ($r^2 = 0.476$, $p < 0.01$) (Fig. 4b). It is also worthy to note that soil enzyme activities showed no obvious change with time. This indicates that turf age had no significant effects on soil enzyme activity, though we detected relatively lower activity of soil enzymes in older turfs.

2.7. Correlations between soil parameters and grass growth

To understand better the relationship between soil properties and DOC/DON, we performed Pearson's correlation analysis on soil properties, turfgrass biomass, DOC and DON (Table 4). We used average values of the three soil depths for soil C and N. There were very significant negative correlations between soil bulk density and water content ($p < 0.01$), as well as turfgrass biomass and TDN ($p < 0.05$). On the other hand, we found positive correlation between bulk density and SOC density ($p < 0.05$). Soil TDN had significant correlation with soil NO_3^- and DON ($p < 0.01$), while DOC correlated significantly with DON ($p < 0.05$). None of the physical properties including bulk density and water content, and grass biomass had any significant effect on soil DOC and DON.

We found significant correlations between soil enzyme activities, grass biomass and soil properties in urban turfs (Table 5). Specifically, grass biomass was significantly correlated with urease ($p < 0.05$) and β-glucosidase ($p < 0.01$) activities, while soil NO_3-N was negatively correlated with β-glucosidase ($p < 0.05$) and protease ($p < 0.05$) activities. A significantly negative correlation was found between soil TDN and β-glucosidase activity ($p < 0.05$).

PCA on soil physical and chemical properties, soil enzyme activities and grass biomass C density revealed a scattered distribution for two soil depths 0–5 cm and 5–10 cm (Fig. 5) in the studied turfs. For 0–5 cm, the first principal component (PC1) accounted for 21.5% and the second accounted for 14.5% of the variance (Fig. 5a). PC1 was accounted mostly by TDN, β-glucosidase activity, NO_3-N, pH and urease activity, while PC2 was bulk density, SOC and soil moisture. Similarly, for 5–10 cm, PC1 accounted for 26.1% and PC2 accounted for 18.5% of the variance (Fig. 5b). PC1 was accounted mostly by TDN, grass biomass C density, NO_3-N, NH_4-N, DON and SOC, while PC2 was β-glucosidase activity, urease activity, soil moisture and bulk density. The biplot of PCA clearly identified two groups of turf sites from Hong Kong and Shenzhen, for both depths (Fig. 5).

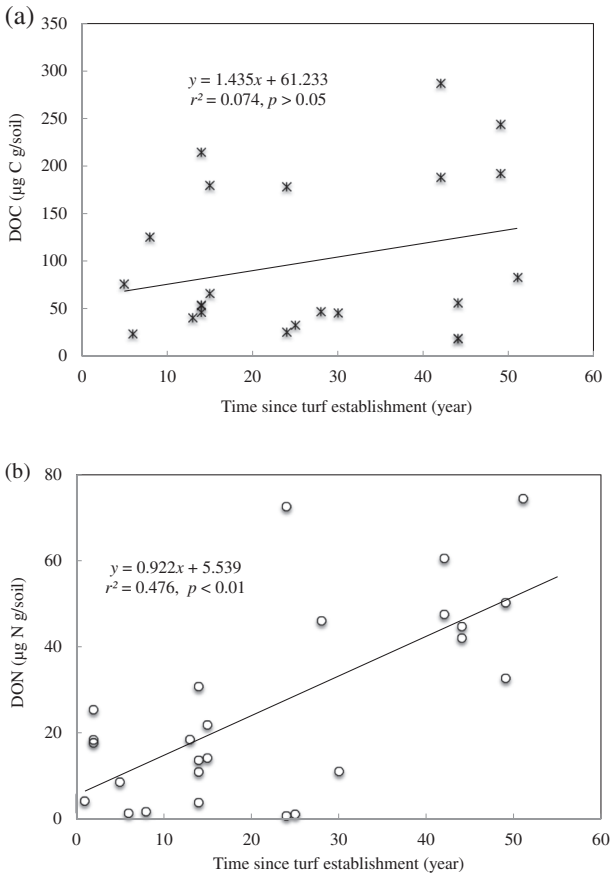


Fig. 4 – DOC (a) and DON (b) concentrations in soils (0–15 cm) in the studied turfs with different ages (3 replicates for each sample, r^2 indicates coefficient of determination).

3. Discussion

Regarding the role of turfgrasses in DOC and DON cycling in urban turfs, grass biomass was negatively correlated with soil TDN, which served as a determining factor in governing DON content in soil. In addition, soil DON was significantly correlated DOC, and therefore grass biomass could influence both soil pools of DON and DOC. We further showed that grass biomass was significantly correlated with urease and β-glucosidase activities in soil. On the other hand, grass biomass varied dramatically among species. *A. compressus* and *Z. matrella* were the two dominant species in the studied turfs, and their biomass differed significantly ($p < 0.05$, Table 3), indicating grass species plays a role in regulating both DOC, DON and soil enzyme activities. These results confirm the notion that the biological factor, *i.e.*, grass species, determines the soil biochemical and physical properties that regulate the release and retention of DOC and DON (Neff and Asner, 2001; McDowell, 2003; Khalid et al., 2007). Consistently, Freeman et al. (2004) proposed a mechanism that net primary productivity (NPP) was driving DOC release, which was more sensitive to atmosphere

Table 4 – Correlation coefficients between soil physical and chemical properties and grass biomass.

	Bulk density	Water content	SOC density	Grass AGB	DOC	TDN	NH_4-N	NO_3-N	DON
Bulk density	1.000								
Water content	−0.489 **	1.000							
SOC density	0.408 *	−0.121	1.000						
Grass AGB	−0.201	0.138	−0.014	1.000					
DOC	-0.038	0.033	0.053	−0.350	1.000				
TDN	0.116	0.200	0.302	−0.445*	0.352	1.000			
NH_4	0.286	−0.106	0.153	−0.313	0.108	0.352	1.000		
NO_3	0.051	0.190	0.218	−0.299	0.153	0.809 **	0.269	1.000	
DON	0.082	0.120	0.212	−0.327	0.394 *	0.618 **	0.073	0.051	1.000

AGB for aboveground biomass;
* Denotes significant correlation at $p < 0.05$;
** Denote significant correlation at $p < 0.01$.

Table 5 – Correlation coefficients between soil enzyme activities, soil properties and grasses biomass in urban turfs.

	Urease	β-glucosidase	Cellulase	Protease
Grass biomass	0.445*	0.532**	0.013	0.001
Soil bulk density	0.012	−0.185	−0.056	0.184
Soil water content	0.019	0.314	0.314	−0.017
Soil organic C	0.165	−0.172	0.127	0.356
Soil DOC	−0.263	−0.097	−0.102	−0.260
Soil TDN	−0.072	−0.393*	0.040	−0.175
Soil NH_4	0.044	−0.001	0.128	−0.083
Soil NO_3	−0.052	−0.467*	−0.050	−0.406*
Soil DON	−0.047	−0.069	0.083	0.158

* Denotes significant correlations at $p < 0.05$;
** Denote significant correlations at $p < 0.01$.

CO_2 than to warming or hydrological changes. However, in contrast to recent reports on DOC and DON in natural settings (McDowell and Likens, 1988), managed urban turfgrass system appeared to follow different rules with regard to DOC and DON. While low anthropogenic N inputs in forest ecosystems could elevate N exports (Cairns and Lajtha, 2005), application of N-fertilizer has been reported to be a factor in determining DOC export in managed grassland, largely as a result of an increase in dry matter production (McTiernan et al., 2001). On the other hand, DOC export decreases due to artificial drainage and its adsorption to soil surfaces.

Soil TDN had also significant correlation with soil NO_3^- and DON. This is perhaps because the concentration of NH_4 in soil was underestimated, due to the use of water for soil sample extraction (Jones and Willett, 2006), which results in NO_3^- and DON accounting for most of the TDN. High SOC did not necessarily lead to high DOC or DON, as no significant relationship was found in our study. However, a significant correlation between DOC and DON was observed, indicating that the two were strongly linked. The interaction between their dynamics needs to be confirmed in future study.

We also observed good correlations between grass biomass and urease and β-glucosidase activities (Table 5). Consistently, soil enzyme activity is related to vegetation (Waldrop et al., 2000; Sinsabaugh et al., 2002; Caldwell, 2005). Both microbial biomass and enzyme activity tend to increase at the rhizosphere, especially with the extensive root system of grasses (Bandick and Dick, 1999). A few studies (Ajwa et al., 1999; Henriksen and Breland, 1999; Michel and Matzner, 2003) have reported that the activity of different cellulases (endocellulase, exocellulase and β-glucosidase) is favored by the increase in the availability of mineral N in prairie and agricultural soils. Fenner et al. (2005) also proposed that C cycling-related soil enzymes such as phenol oxidase and β-glucosidase were crucial in DOC mobilization and C exports in peat land. Allison et al. (2010) presented a microbial-enzyme model to illustrate the role of enzymes in SOC decomposition, with microbial biomass mediating the conversion of SOC to DOC through the production of extracellular enzymes.

Surprisingly, we observed that soil NO_3-N was negatively correlated with β-glucosidase and protease activities. A higher level of β-glucosidase indicates higher organic matter quality and nutrient availability, and rapid mineralization (Sinsabaugh and Moorhead, 1997). Consistently, protease has been considered a rate-limiting factor in soil organic N solubilization and mineralization (Nunan et al., 2000; Allison and Vitousek, 2005), thus resulting in NO_3^- leaching, which is readily available to plants while easily lost from the soil.

Previous studies have shown that enzyme activity as well as C and N dynamics could be affected by the particle size distribution of the soils. Kandeler et al. (1999a) reported that C and N contents varied with soil particle size distribution, which were strongly influenced by management practices such as organic amendment application. They also observed the predominance of xylanase and urease in different particle size fractions. Consistently, xylanase activity was reported to increase gradually with diminishing particle size (Poll et al., 2003) and the distribution of enzymes between fractions varied with the enzymes (Marx et al., 2005). Furthermore, a study on restored grassland soils showed the difference in enzyme production and C turnover between different fractions, which was faster in particulate organic matter fractions but slower in mineral-dominated fractions (Allison and Jastrow, 2006).

4. Conclusions

We demonstrated that vegetation was important in controlling soil enzyme activity, with grass biomass and the activities of urease and β-glucosidase significantly correlated. Vegetation also palys a role in soil organic C and N pools as significant correlations were found between grass biomass and soil TDN, and between DOC and DON in soil. In addition, soil enzyme activities, C and N showed obvious vertical variations and temporal changes, which decreased with soil depth. DON increased with turf age, while DOC and soil enzyme activities were not altered by turf age.

Soil enzymes could become sensitive to turfgrass maintenance practices in terms of grass species and soil properties, particularly the distribution of particle size. In particular, β-glucosidase activity correlated well with grass biomass, while negatively correlated with soil TDN and NO_3-N. Protease activity also showed a negative correlation with soil NO_3-N. It is argued that soil enzymes such as β-glucosidase correlated well with soil DOC and DON stock, which is critical to soil C flux and cycle. However, soil texture and management practices are also critical factors in regulating both soil C and N dynamics and soil enzymatic activities. Therefore, the stock and export of soil organic matter should be engineered based on the selection of grass species and soil types, in combination with fertilization and irrigation regimes, as well as other maintenance practices. This could help us better understand the contribution of turf management and maintenance regarding C and N dynamics in urban soils.

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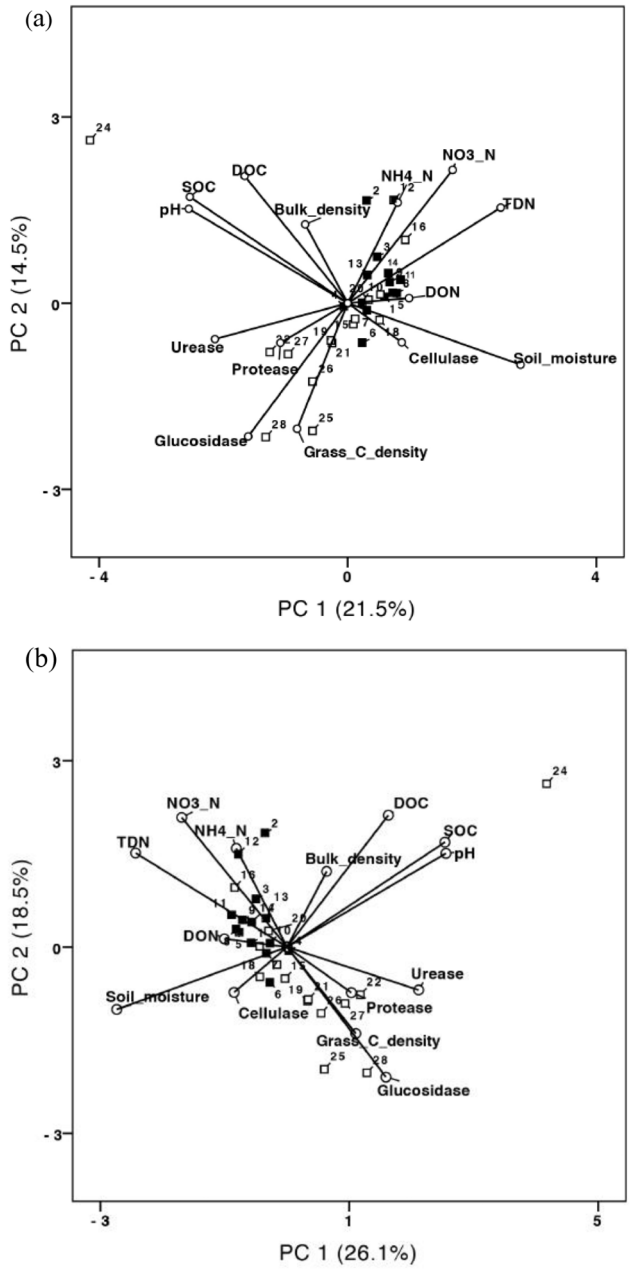


Fig. 5 – Biplot of principal component analysis (PCA) of soil physical and chemical properties, and soil enzymatic activities at 0–5 cm (a) and 5–10 cm (b) for soils in the studied turfs. SOC: soil organic carbon; DOC: dissolved organic carbon; TDN: total dissolved nitrogen; DON: dissolved organic nitrogen; NO_3N: extractable NO_3-N; NH_4N: extractable NH_4-N; Grass C density: Grass biomass carbon density; Open squares denote turfs sites in Hong Kong with number 1–14 and solid squares denote.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2017.04.006.

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