Changes of Microbial Community Associated with Construction Method and Maintenance Practise on Soil Profile in Golf Courses

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지반 조성과 관리방법에 따른 골프장 토양내 미생물 군집의 변화

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ABSTRACT

The construction procedures and artificial turf maintenance program on golf course definitely influence on the distortion of its environment. Soil microbial communities in soil profile were affected directly by those practises on turf areas. In Jeju island, the environmental impact assessment has been required to apply the first quality class granular activated carbon(GAC), which has a high absorbent character to agricultural chemicals, on the soil profiles of golf green system to reduce the pesticide leaching to ground water. This research was carried out to analyze the changes of microbial communities and chemical properties on soil profiles where GAC had been applied at the construction stage at two golf courses in Jeju. The changes of soil microbial population and chemical properties associated with construction methods of soil profile and agrochemical management program were analyzed by monthly at the surface and sub-soil profiles during April through October, 2007. The total numbers of bacteria and fungi, soil moisture content, soil physio-chemical properties were measured on greens and fairways of the both golf courses with different GAC treatment on the green and fairway soil profiles.

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The results showed that GAC had positive effects on the water holding capacity, pH and EC, however, it did not improved the holding capacity of available nutrients NO_3^-,NH_4^+, and phosphorus by its sorption phenomenon. In microbial count test, the total numbers of bacteria and fungi showed a great variation during sampling dates.

That may directly relate to the agrochemical application, however, the ratio of total bacterial number versus total fungus number showed a constant value on a sub-soil of 15~30cm depth. Thus, the construction method of GAC in soil profile, and application of fertilizer and pesticide, both impacted on the changes of microbial population. It's means that the construction method of soil profile and turf management using agro-materials might greatly affect on the turfgrass culture and the environment of golf course.

Key words: Jeju golf course, chemical properties, microbial properties, activated carbon

INTRODUCTION

The Jeju island has very poor in surface water resource, however the ground water is very abundant because of a high vertical hydraulic conductivity of 3-28 $m \cdot day^{-1}$. Moreover, it shows very little rate of the surface runoff caused by a rapid rainfall infiltrates(Kim et al., 2003). The environmental impact assessment has been required to apply the first quality class granular activated carbon(GAC), which has a high absorbent character to agricultural chemicals, on the soil profiles of golf green system to reduce the pesticide leaching to ground water.

In recent study by Lee et al.(2006) assessed the application of agricultural chemical absorption capability of GAC and charcoal in the golf course green in Jeju. The result showed that there was no difference in using two materials in a Smithion absorbent test. Using such a GAC or charcoal might impact on soil microbe communities but little investigations about the bio-chemical properties of soil types of Jeju has been reported.

The application rate and frequency of pesticides and fertilizers mainly depends upon the decisions from superintendent and a legislative or labeling restriction of the chemicals (Mark et al., 2007). Those various management practices might affect the soil microbes community in turf areas. Many researches conducted by agrochemical companies have been showed that soil conditioners have promoted the activity of bacteria and fungi and eventually improved the turfgrass growth(Hagley, 2002; Mueller and Kusow, 2005). The soil microbial biomass has a important roles on the organic matter degradation, nutrient supply, mycorrhizal colonization, and disease occurrence and suppression, thus those activities directly connected with turf environments(Alexander, 1977; Nelson, 1992, 1994; Gregorich et al., 1994; Turgeon, 1996). Soil microorganisms play a crucial role in the cycling of almost all major plant nutrients and the energy flow of either natural or anthropogenically altered soil(Smith and Paul, 1990). Despite of its known importances, there is little information on the amount and significance of microbial biomass in golf greens. Populations of total fungi and total bacteria, as well as selected microbial groups, have been enumerated in golf greens by plating onto various media(Mancino et al., 1993; Liu et al., 1995; Elliott and Des Jardin, 1999a, 1999b). Research has also shown that fungal and bacterial ratios vary in response to forest fertility, with the relative abundance of bacteria increasing in response to increased fertility(Pennanen et al., 1999). The proportion of active in soil with lower nutrient concentrations(Gronli et al., 2005; Wallenstein et al., 2006). Nutrient retention and dynamics in sand-based or sand and GAC-based turfgrass systems have not been well documented with time. Understanding nutrient status and dynamics classified by depth is important, since it impacts management practices and turfgrass health.

This research was carried out to analyze the changes of microbial communities and chemical properties on soil profiles where GAC had been applied at the construction stage at two golf courses in Jeju. The changes of soil microbial population and chemical properties associated with construction methods of soil profile and agrochemical management program were analyzed by monthly at the surface and sub-soil profiles during April through October, 2007.

MATERIALS AND METHODS

Site description and soil sampling

This study was conducted at the Lake Hills Jeju Country Club(A golf course) and Cypress Golf & Resorts(B golf course) from April to October 2007 in Jeju island(126°08′~126°58′E, 33°06′~34°00′N). The sand-base of the soil profiles contained partially with zeolite, peat moss and activated carbon(GAC or charcoal). The greens in A golf course contained the mixture of 3% zeolite and peat moss, 3 cm thickness charcoal carbon at 30cm depth. The fairways of B golf course contained the mixture of 3% zeolite and peat moss without carbon materials. The greens and fairways of the B golf course contained the mixture of 3% peat moss, 400g • m^{-2} zeolite and GAC.

At each golf course, 9 randomly selected greens and fairways were sampled. Each soil core was taken with an auger(108mm diameter) to 0-15 cm and 15-30 cm depths of soils on April, June, August and October, 2007 with three replications.

The bentgrass plots received moderate fertilization, approximately 40-30-40 g N-P_2O_5-K_2O per yr with a complete chemical fertilizer, mostly urea and ammonium forms of the N. Pesticides including fungicides, insecticides and herbicides were applied for a preventative or post-emergency purposes during the growing seasons.

Chemical property of soil

The samples were air-dried, ground, and passed through a 2mm sieve. The pH and electrical conductivity(EC) of the soil solution were measured in suspensions of soil samples in water using a 1:5 soil-water ratio. The concentrations of NO_3^- and NH_4^+ in the soil were measured by using a spectrophotometer(KA-P, Soiltek). NH_4^+ was extracted from soil with ionized water. Pipette an aliquot(normally < 3mL, not to exceed 5mL) of the soil extract into a 25mL volumetric flask. Add 1mL of EDTA reagent, and swirl the flask to mix the contents. Then add 4mL of salicylate-nitroprusside reagent, swirl the flask, and bring the volume to approximately 20mL with deionized water. Add 2mL of buffered hypochlorite reagent, immediately bring the volume to 25mL with deionized water, and thoroughly mix the contents of the flask. Place the flask in a water bath at 37°C to develop the color. After 30 min, remove the flask from the bath, allow the solution to cool to room temperature for 10 minutes, and measure its absorbance at 667nm against a reagent blank solution. Calibrate absorbance measurements by analysis of standards containing 10ppm of NH_4^+-N. To prepare these standards, pipette into seven 50mL volumetric flasks the same volume of 2 M KCl as the aliquot of soil extract taken for analysis, and add 0, 1, 2, 3, 5, and 7 mL of the working standard$(NH_4)_2SO4$ solution. Then carry out color development, and measure the absorbance by the procedure described for analysis of the extract(D.L. Sparks 2005, 5: 1152-1155).

The available P of soil was measured by Nelson et al. extraction procedure(1953) and determination of ascorbic acid method with a spectrophotometer at wavelength 880nm(Sparks, 2005). Transfer an aliquot of sample or P standard solution that contains 2 to 4μg P to a 50-mL volumetric flask. Dilute with deionized water to about 25mL, and add 8mL of mixed reagent. Dilute the solution to volume and mix well. Measure the absorbance at 880nm after 10 min.

Biological property of soil

Sampling soil bags were transported on ice pack to lab and sampled for microbial plat counts. Soil microorganisms were extracted by shaking 5g of the sampling soil in

45mL of sterile distilled water. The suspension was diluted with distilled water 10-fold serial dilutions triplicate for total bacterial number and duplicate for total fungal number. For total bacterial number of soil, 0.1mL suspension was plated out on Plate Count Agar(PCA, BD). For total fungal number of soil, 0.1mL suspension was plated out on Rose Bengal Agar(RBA, Sigma). The plates were incubated at 32 ℃ in the dark. Colonies were counted after 1 day. Daga from triplicate readings were expressed as colony forming units(CFU)g^{-1} dry soil.

Statistical analysis

Statistical analyses were conducted with SAS 6.12(SAS Institute, Inc., Cary, NC). Analysis of variance (ANOVA) and least significant difference(LSD) were performed(α =0.05).

RESULTS AND DISCUSSION

Chemical properties

Table 1 and 2 show that chemical properties were observes to be significant in at least one way interaction involving soil depth and golf course. The management of sampling two golf course was different, but similar with monthly chemical alteration. Soil moisture was not different among sub-soils except for A golf course fairway sub-soil. Fairway of B golf course sub-soil has high water content compared to A golf course because no differences in sub-soil moisture were observed among treatments at the 15.9~20.8% from June to October. It related to root growth. Table 3 showed that the chemical property NH_4^+ of green sub-soil is not significantly different by month. However, the variance of NO_3^- and P_2O_5 is associated with each month variable.

Table 1. The chemical properties(moisture, pH, and EC) of fairway soils for A and B golf courses in 2007.

	Surface soil				Subsoil			
	Apr	Jun	Aug	Oct	Apr	Jun	Aug	Oct
A golf course								
Moisture(%)	7.3^b	8.9^b	17.8^a	18.5^a	10.2^c	9.4^c	13.9^b	18.9^a
pH	6.36^b	6.47^b	6.37^b	7.15^a	6.41^c	6.59^b	6.39^c	6.91^a
EC(dS · m^{-1})	0.003^c	0.003^{bc}	0.006^a	0.003^b	0.002^b	0.002^b	0.004^a	0.002^b
B golf course								
Moisture(%)	9.2^b	10.7^b	13.6^b	17.7^a	-	15.9^a	20.8^a	18.9^a
pH	6.08^c	6.52^b	6.27^{bc}	6.74^a	-	6.31^b	6.16^b	6.57^a
EC(dS · m^{-1})	0.004^a	0.003^b	0.004^a	0.002^c	-	0.002^a	0.003^a	0.002^b

Values with different superscript letters in a row of each surface and sub-soils are significantly different by least significant difference(α =0.05) test.

Table 2. Effects of the chemical properties(NH_4^+, NO_3^- and P_2O_5) of green soil for A and B golf courses in 2007.

	Surface soil				Sub-soil			
	Apr	Jun	Aug	Oct	Apr	Jun	Aug	Oct
A golf course								
NH_4^+(mg/kg)	0.69^a	0.26^{bc}	0.18^c	0.38^b	-	0.15^a	0.10^a	0.17^a
NO_3^-(mg/kg)	14.7^a	6.6^{ab}	0.4^c	4.2^b	-	8.3^a	0.3^b	4.4^c
P_2O_5(mg/kg)	17.1^a	6.2^b	0.3^c	3.6^{bc}	-	4.6^a	0.1^c	1.4^b
B golf course								
NH_4^+(mg/kg)	0.53^a	0.09^b	0.08^b	0.11^b	-	0.08^a	0.12^a	0.11^a
NO_3^-(mg/kg)	11.3^a	7.9^b	0.12^d	5.05^c	-	8.39^a	0.12^c	4.44^b
P_2O_5(mg/kg)	16.1^a	3.3^b	0.1^c	1.5^{bc}	-	2.6^a	0.1^c	1.1^b

Values with different superscript letters in a row of each surface and sub-soils are significantly different by least significant difference(α=0.05) test.

Table 3. Effects of the chemical properties (NH_4^+, NO_3^-, and P_2O_5) of fairway soil for A and B golf courses in 2007.

	Surface soil				Sub-soil			
	Apr	Jun	Aug	Oct	Apr	Jun	Aug	Oct
A golf course								
NH_4^+(mg/kg)	0.9^a	0.3^b	0.4^b	0.8^a	-	0.2^a	0.2^a	0.3^a
NO_3^-(mg/kg)	10.5^a	7.4^a	0.4^b	5.0^a	-	10.4^a	0.3^c	4.8^b
P_2O_5(mg/kg)	21.3^a	4.2^b	0.4^c	3.6^{bc}	-	3.3^a	0.2^c	1.6^b
B golf course								
NH_4^+(mg/kg)	1.5^a	0.3^{bc}	0.1^c	0.3^b	-	0.2^a	0.1^a	0.1^a
NO_3^-(mg/kg)	10.6^a	8.4^a	0.1^b	8.1^a	-	8.0^a	0.1^c	5.0^b
P_2O_5(mg/kg)	22.3^a	4.2^b	0.2^d	2.2^c	-	2.8^a	0.1^c	1.3^b

Values with different superscript letters in a row of each surface and sub-soils are significantly different by least significant difference(α=0.05) test.

The A golf course green which paved GAC floor in the 30cm soil depth and B golf course green surface soil variation patterns are similar. There is no fertilization capacity difference between sand mixed with GAC and sand only base soils. At fairways of the A and B golf course, the patterns of each chemical measured components is alike that is not same we think. The GAC would not hold soluble nutrients and not increase the nutrient concentration of sand GAC mixed soil. Concentrations of phosphorus in green on April the greatest value of P for surface soil of green and fairway in A golf course and surface soil green and fairway for B golf course were 17.1mg • kg^{-1}, 21.3mg • kg^{-1}, 16.1mg • kg^{-1}, and 22.3mg • kg^{-1}, respectively.

Biological properties

Bacterial and fungal numbers are different according to Soil order, depth, cultivation type. Bacteria can increase their populations quickly in responds to favorable changes in soil environment and food availability.

Table 4. Mean populations of bacteria and fungi of green soil for A and B golf courses in 2007.

	Surface soil					Sub-soil				
	Apr	Jun	Aug	Oct	LSD	Apr	Jun	Aug	Oct	LSD
A golf course										
Total bacteria (x 10^5cfug^{-1}soil)	-	-	0.54^b	3.55^a	2.49	-	-	0.54^a	1.88^a	2.07
Total fungi (x 10^3cfug^{-1}soil)	0.76^a	-	2.18^a	3.05^a	1.67	0.14^b	-	2.94^a	0.52^b	2.28
Fungi/Bacterial ratio	-	-	0.05^a	0.02^b	0.03	-	-	0.096^a	0.005^a	0.09
B golf course										
Total bacteria (x 10^5cfug^{-1}soil)	-	1.04^a	3.06^a	4.38^a	2.54	-	1.82^a	0.46^a	4.68^a	5.99
Total fungi (x 10^3cfug^{-1}soil)	-	2.13^a	0.26^b	2.74^a	1.61	-	2.74^a	0.09^b	1.85^{ab}	2.05
Fungi/Bacterial ratio	-	0.026^{ab}	0.001^b	0.077^a	0.73	-	0.049^a	0.004^b	0.042^{ab}	0.03

Values with different superscript letters in a row of each surface and sub-soils are significantly different by least significant difference(α=0.05) test.

Generally, however, in case of the same soil order, populations of bacteria at surface soil(0～10cm) are thousands times more than fungi and the gap of them was reduced under 10cm soil(Brady, 2002). The appearance of total fungi change in the B golf course green soil mixed with GAC was similar with fungi/bacterial ratio. The ratio of fungi to bacteria ranged from 0.005～0.09 in A golf course and 0.001～0.07 in B golf course. These low ratios indicate that fungi were less abundant than the bacteria in the extracted samples. The fairway's fungi/bacterial ratio was different(Table 5). The fungi/bacterial ratio in B golf course was lower than A golf course. Based on LSD analysis, there was no significant change in fungi/bacterial ratio in A golf course surface soil. The changes of the total fungi and fungi/bacterial ratio were quite similar except the A golf course fairway surface soil. Lower fungi/bacterial ratio was observed in B golf course surface soil than sub-soil. This fungi/bacterial ratio is likely to be related to shifts in nutrient concentration and soil depth.

In accordance with multiple surveys of fungal to bacterial biomass, we observed that fungal/bacterial ratios were greater in forest soils than in agricultural or grassland soils(Bailey et al., 2002; Bossuyt et al., 2001; Hogberg et al., 2007; Treseder, 2004). Increased fungi in aggrading soil systems may be a mechanism for increasing whole-soil C storage, because fungi (i) are unique in their ability to translocate and

Table 5. Mean populations of bacteria and fungi of fairway soil for A and B golf courses in 2007.

	Surface soil					Sub-soil				
	Apr	Jun	Aug	Oct	LSD	Apr	Jun	Aug	Oct	LSD
A golf course										
Total bacteria (x 10^{5}cfug^{-1}soil)	-	-	8.10^{a}	2.99^{a}	15.0	-	-	1.00^{b}	3.80^{a}	
Total fungi (x 10^{3}cfug^{-1}soil)	1.58^{b}	-	10.58^{a}	5.12^{b}	2.20	0.20^{b}	-	5.28^{a}	0.90^{b}	2.64
Fungi/Bacterial ratio	-	-	0.05^{a}	0.05^{a}	0.07	-	-	0.13^{a}	0.01^{b}	
B golf course										
Total bacteria (x 10^{5}cfug^{-1}soil)	-	2.24^{b}	5.06^{a}	0.72^{b}	1.87	-	3.36^{a}	1.08^{b}	1.46^{b}	0.42
Total fungi (x 10^{3}cfug^{-1}soil)	-	2.23^{a}	0.40^{b}	1.69^{ab}	1.54	-	0.59^{a}	0.14^{a}	1.15^{a}	1.23
Fungi/Bacterial ratio	-	0.029^{a}	0.002^{b}	0.034^{a}	0.03	-	0.009^{a}	0.001^{a}	0.028^{a}	0.04

Values with different superscript letters in a row of each surface and sub-soils are significantly different by least significant difference(α=0.05) test.

utilize spatially separated nutrient resources(e.g., Frey et al., 2003), (ii) are believed to respire more efficiently than bacteria(e.g., Holland and Coleman, 1987), and (iii) are composed of more complex recalcitrant compounds(e.g., Guggenberger et al., 1999). Further, even when plant community composition is similar, reduced agricultural disturbance can increase fungal dominance(Victoria, 2005).

Relationships between chemical properties and biological properties

Table 5, 6 show that the correlation between soil chemical properties(pH, EC, NH_4^+, NO_3^-, and P_2O_5) and total bacterial number(TB), total fungal number(TF) and F/B ratio of in the A, B golf courses green and fairways. In the A golf course, soil pH was negatively correlated with EC value and NH_4^+. Generally, soil pH is negatively correlated with P_2O_5 in pH 4 to 7 range. But, in this study, the result was not related. The pH range is narrow, about pH 6 to 7 in A and B golf course.

P_2O_5 was significantly positively correlated with NO_3^-($p<0.001$). P_2O_5 solubilization is influenced by the management of NO_3^- concentration. It is also generally accepted that TB is influenced chemical properties, especially, N, P and pH. The correlation TB with chemical properties is not more influenced than TF of this study. F/B ratio is also influenced by TF. Lovell et al.(1995) suggested that differences in soil microbial biomass were associated with changes in community structure, in particular in the relative proportion of bacteria and fungi. FB ratio was negatively correlated with N concentration-NO3- of A golf course and increased with total fungi. In number of other successional systems F/B ratios continue to increase with time.

Table 6. Pearson linear correlations between soil chemical properties(pH, EC, NH_4^+, NO_3^- and P_2O^5) and total bacterial number(TB), total fungal number(TF) and F/B ratio of in A golf course in 2007.

	pH	EC	NH_4^+	NO_3^-	P_2O_5	TB	TF	F/B ratio
pH	1.00	-0.37***	0.44***	0.13	-0.03	0.13	0.01	-0.18
EC		1.00	-0.03	-0.42***	-0.16	-0.02	0.36***	0.24
NH_4^+			1.00	0.09	0.34***	0.12	0.20	0.01
NO_3^-				1.00	0.39***	0.02	-0.23*	-0.27*
P_2O_5					1.00	0.01	-0.16	-0.21
TB						1.00	0.16	-0.13
TF							1.00	0.45***
F/B ratio								1.00

*Significance at the 0.05 probability level.
**Significance at the 0.01 probability level.
***Significance at the 0.001 probability level.

Pennanen et al.(2001) found increased relative abundance of fungi with time in a primary successional system, and suggest that fungal biomass increased in response to the accumulation of organic matter and increasing C/N ratio of the plant material.

국문 요약

골프장은 인공적으로 건설되어 지고 관리되므로 당연히 환경의 변화가 생성된 곳이므로 건설방식이나 관리 방법에 따라 코스 내 토양 미생물 군집 구조의 특이성이 있을 것이다. 현재 제주도에서는 농약 용탈 저감방안으로 골프코스 그린지반구조에 농약 흡착층을 설치하고 그 재료로 흡착성능이 우수한 입상 활성탄 1등급을 사용할 것을 환경 영향평가서에 명시하고 있다.

본 실험은 활성탄이 처리된 제주도 A, B 골프장을 대상으로 토양에서의 화학적 특성변화와 미생물 군락변화를 분석하였다. 2007년 4월, 6월, 8월 10월에 걸쳐서 총 박테리아 수, 총 곰팡이 수, 수분함량 및 토양 이화학성(pH, EC, NO3-,NH4+ 및 P2O5)의 변화를 그린과 페어웨이에서 깊이별(표토:0-15cm, 심토: 15-30cm)로 조사되었다.

그 결과, 활성탄의 시용이 수분 보유 능력, 토양산도, 전기전도도에 긍정적인 영향을 보였으나, GAC의 물질의 수착 능력에 의하여 유효 양분들을 많이 보유하는 데 도움이 될 것이라 예상하였으나, 본 실험에서는 가용성 질산과 암모늄, 및 인산의 농도를 높이는 효과는 없었다. 토양 미생물 실험에 있어서는 총 박테리아 및 총 곰팡이의 시기적 변화가 다양성을 보였다. 이러한 현상은 농약의 시용에 따라 직접적인 관련이 있을 것으로 예상되었으나, 총 곰팡이/총 박테리아 비(F/B ration) 는 활성탄을 혼합한 토양 심토(15-30cm)에서 일정한 수치를 유지하였다. 따라서, GAC 토양층의 설치 방법이나 시비와 시약 등이 미생물군집의 변화에 영향을 주며 이는 농약의 시용이 잔디관리에 큰 변수로 작용한다는 것을 시사한다.

주요어 : 제주골프장, 토양 화학성, 토양 미생물성, 활성탄

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