Influence of Soil Management Practices on β-*Proteobacteria* Ammonia-Oxidizing Communities in Turf-Covered Aridisols

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Abstract

We investigated the activity, identity, and abundance of ammonia oxidizing bacteria (AOB) in turf-covered aridisols from four golf courses receiving different fertilizer and irrigation regimes. The golf courses varied in age from 6-24 y and received tertiary treated water, groundwater, or Colorado River water and mixtures of ammonium, nitrate and/or urea fertilizer. The oldest soil had ca. twice the total organic carbon as the other soils. Rates of potential nitrification activity (PNA) generally increased with increasing duration of management, although the oldest golf course had lower PNA than expected, likely due to high soil salinity from long-term irrigation with tertiary treated water. Non turf-covered soils from the courses (i.e. sand traps) served controls with low rates of PNA. All soil samples generated PCR products using primers specific for β-*Proteobacteria* AOB. Different representatives of *Nitrosomonas* and *Nitrosospira* species were supported by the soils as determined by DGGE and DNA sequencing. Population abundance was lower in soils irrigated with tertiary treated water as determined by real-time PCR.

Introduction

Turfgrass environments are among the most rapidly increasing managed ecosystems and were recently documented as potentially significant sinks for atmospheric carbon dioxide. Golf courses, a type of turfgrass environment, within the same geographic range have identical soil composition and texture although each receive different fertilizer and irrigation regimes as determined by the course managers. Irrigation of turf grasses with wastewater effluent is becoming an inexpensive option to fresh or groundwater irrigation; however, irrigation with wastewater effluent may have possible adverse side effects as these waters often contain high levels of solutes (*i.e.*, salts, organic carbon and metals), and may provide inocula of undesirable microorganisms. Hence, the application of wastewater effluent could significantly alter nutrient cycling and, in turn, negatively affect turf and plant health.

In-depth analysis of the microbial ecology of turf soils irrigated with treated wastewater is necessary to gain a better understanding of the potential effects on soil health and plant production. The ammonia-oxidizing bacteria are responsible for the oxidation of ammonia to nitrite and effectively compete for fertilizer nitrogen with plants. The goal of this study was to examine the impact of different fertilization regimes and irrigation water sources on the activity, population structure, and abundance of AOB in four golf course soils.

Materials and Methods

Samples: Five 200 g soil samples were taken from both the rough and a sand trap at each of 4 golf courses located in the Coachella Valley, CA. HP=Heritage Palms, IR=Indian Ridge, PV=Palm Valley, and SR=Santa Rosa. Three liters of water were collected from individual sprinkler heads.

Chemical analyses: Soil pH, ammonium and nitrate content, total cation and anions, organic carbon, CEC, and potential nitrification activity were determined using standard methods [1, 2].

Microbial analyses: Total soil DNA was extracted using commercial kit.

AOB composition was determined using DGGE of the products from nested PCR of CTO189f-AB/C and CTO654r [3] and 341f-GC and 518r [4].

AOB abundance was determined by real-time PCR, using CTO189f [3] and RT1r [5] primers and SYBR-Green as indicator.

References

[1] Thomas, G.W. 1996. Methods of Soil Analysis, Part 3. pp. 475-490.
[2] Mintie *et al.* 2003. Appl. Environ. Microbiol. 69:3129-3136.
[3] Kowalchuk *et al.* 1997. Appl. Environ. Microbiol. 63:1489-1497.
[4] Muyzer *et al.*, 1993. Appl. Environ. Microbiol. 59: 695-700.
[5] Hermansson & Lindgren, 2000. Appl. Environ. Microbiol. 67:972-976.

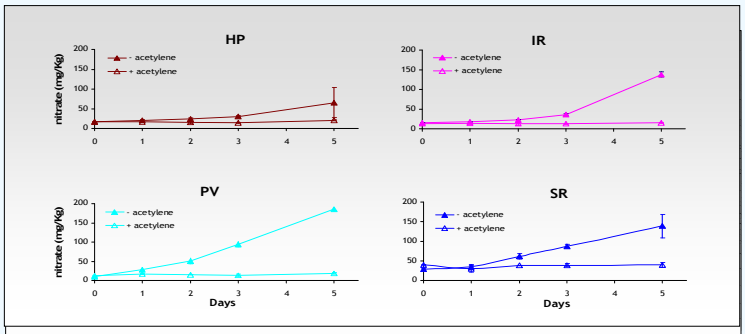
Results & Discussion

Chemical Properties of the Soil and Irrigation Water Samples

Site	Irrigation Source	Fertilizer -N	Applicn (lb-N/1000ft²)	Age (Y)	PNA (mg NO_2^-/Kg soil/day)	%TOC	CEC (meq/ 100g)	pH	NH_4^+ (mg/ Kg)	NO_3^- (mg/ Kg)	Mg (µeq/ Kg)	Ca (µeq/ Kg)	K (µeq/ Kg)	Na (µeq/ Kg)	Cl (µeq/ Kg)
HPR		NH_4^+/NO_3^- /Urea	8	6	10 ± 7.4	2.1	7364	7.4	2.3	5.0	886	1643	352	1304	579
HPS					2.5 ± 1.9	0.8	1630	8.0	1.4	0.8	405	1055	141	805	331
	Colorado River							7.7	0.4	0.01	2559	3599	132	4960	3149
IRR		NH_4^+/NO_3^-	3	10	24 ± 1.3	1.7	4552	7.2	3.7	4.4	449	1130	510	770	367
IRS					-0.1± 0.3	1.7	2055	7.8	1.3	0.4	147	333	145	560	241
	3° treated water							6.9	0.3	8.4	720	2189	324	2850	1932
PVR		NH_4^+/NO_3^- /Urea	3	18	36 ± 1.5	2.5	6791	7.0	3.6	4.2	391	504	295	771	243
PVS					9.0 ± 0.2	0.8	3406	8.0	1.0	0.6	133	490	170	518	172
	Ground water							8.1	0.8	4.4	523	1222	97	1203	228
SRR		NH_4^+/NO_3^- /Urea	1	24	23 ± 5.7	4.3	7813	7.3	4.1	16.0	998	2866	1818	7266	4939
SRS					0.5 ±0.9	1.0	4145	7.7	2.9	1.7	246	898	256	978	481
	3° treated water							8.5	0.4	6.1	715	1956	322	2723	1823

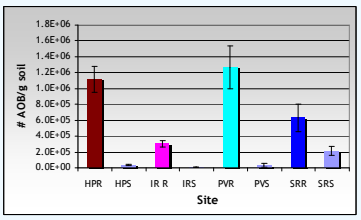
Soil samples have varying amounts of dissolved substances; SR soil had the highest amount of dissolved ammonia and nitrate, cation and anions, and salts in particular. Colorado River water had the highest amounts of salts. (HPR, IRR, PVR and SRR denotes rough soils; HPS, IRS, PVS and SRS, sand traps.

Potential Nitrification Activity

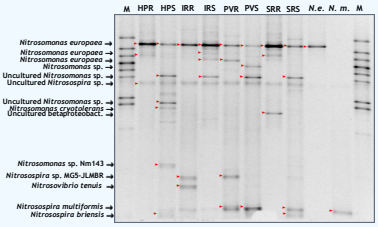


Nitrate accumulation in soil slurries with acetylene (heterotrophic nitrification only) and without acetylene (total nitrification). High potential nitrification rates suggest that ammonia oxidizers were active in all soils. Activity correlated with golf course age, but was low in SR likely due to saline soil. PNA in sand traps were low (Table 1).

Abundance and Community Composition



The number of AOB per gram of soil as measured by real time PCR using SYBR-Green and AOB-specific primers. AOB population was lower in soils receiving tertiary treated water (IR and SR). Population sizes were lower in sand trap than rough soils.



DNA profile after DGGE (8%, acrylamide, 40-60% denaturant). Sequence comparison showed that the DGGE bands were affiliated to *Nitrosomonas* spp., *Nitrosospira* spp. and non-AOB. *N.e., Nitrosomonas europaea; N.m., Nitrosospira multiformis.*

Conclusions

- AOB were active in all of the soils and the activity levels generally correlated with the duration of turf management, with some depression from soil salinity.
- The abundance of AOB was significantly lower in soils irrigated with tertiary treated water relative to other water sources and did not correspond to activity levels.
- *Nitrosospira* spp. and *Nitrosomonas* spp. were present in all of the soils; however each soil supported a different sub-set of AOB.
- Diversity and abundance of AOB in each soil is currently being verified using PCR primers specific for *amoA*.

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