Carbon Sequestration by Nitrifying Microorganisms in Arid Turf Soils

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Introduction

The goal of this study is to examine whether particular management strategies of turfgrass environments located in arid Southern California influence their ability to sequester carbon dioxide. Specifically, we are assessing the effects of nitrogen and irrigation water sources on activities and community structure of free-living, nitrifying, microbial communities.

The nitrifying microbial community is influenced by nitrogen input and controls nitrogen availability to crop plants by consuming ammonia and producing nitrate. Two groups of bacteria comprise the nitrifiers: ammonia- and nitrite-oxidizing bacteria (AOB and NOB, respectively). AOB obtain their energy and reductant for growth via the oxidation of ammonia to nitrite, while NOB oxidize nitrite to nitrate. Both communities are autotrophic and fix CO_2. Additionally, the metabolic activity associated with nitrifying bacteria may also contribute to other environmental concerns such as ground water nitrate contamination and the release N_2O, a noxious greenhouse gas.

We examined samples from four different golf courses located in the Coachella Valley: Indian Ridge, Palm Valley, Heritage Palms and Santa Rosa All four courses have similar textural properties and turf crops but each course has a unique fertilization and irrigation regime (Table 1) allowing for an ideal experimental design. By achieving the research objectives stated below, we anticipate modifications to Turf Best Management Practices where N-fertilizer applications should help to sequester carbon dioxide from the atmosphere and reduce nitrate leaching into the subsurface.

Research Objectives

- V Measure potential nitrification activity of each soil
- V Measure nitrifying community composition
- V Measure $^{14}CO_2$ incorporation into biomass

TABLE 1. Irrigation water and fertilization characteristics of turf in the Coachella Valley

Golf Course	Irrigation Source	EC	pH	Fertilizer-N	Application	Frequency
Indian Ridge	3º treated-H_2O	650	6.9	NH_4^+/ NO_3^-	2.7 lb-N/1000 ft²	annually*
Santa Rosa	3º treated-H_2O	590	8.5	NH_4^+/ NO_3^-/Urea	1.2 lb-N/1000 ft²	annually
Heritage Palms	Canal H_2O	1150	7.7	NH_4^+/ NO_3^-/Urea	8 lb-N/1000 ft²	annually
Palm Valley	Ground H_2O	290	8.1	NH_4^+/ NO_3^-/Urea	3 lb-N/1000 ft²	annually

Soil: Myoma fine sand
Turf: Bermudagrass and Perennial Rye
* Indian Ridge fertilizes during the winter season only
** Santa Rosa 3º treated-H_2O exposed to atmosphere

Materials & Methods

Golf Course Soils

5 200 g soil samples were collected at each site (Table 1)
-3 from the rough and 2 from the sand traps
-Samples were refrigerated or frozen upon collection

Soil Chemical Analyses

Soil pH
-1:1 H_2O & 1:2 0.1M $CaCl_2$
Soil Nitrate and Ammonia Concentrations
-1:1 soil to water extracts
- nitrate and ammonia (Technicon Flow Analyzer)

Potential Nitrification Activity Measurements

-5 g soil : 50 ml NH_4 / PO_4 buffer (pH 7)
-With/Without 1 % (v/v) acetylene
-Measured nitrate concentrations daily
-Measured final pH and headspace acetylene

Molecular Microbial Analyses

Soil DNA Extracts
- 0.5 g composite sample of each soil
- MOBIO Ultra Clean Soil DNA Kit with 2 columns
Control DNA = pure cultures of *Nitrosomonas* and *Nitrosospira*
AOB-specific 16S rDNA PCR
- with βAMO primers (*McCaig et al. 1994. FEMS Microbiol. Let.*)
Restriction Fragment Length Polymorphism
-McCaig PCR product cleaved with restriction enzyme, Alu I

AOB Diversity

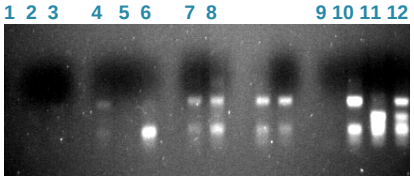

Figure 2. RFLPs of AOB DNA amplified from (lanes 1-3) Heritage Palms, (lanes 4-6) Indian Ridge and (lanes 7 & 8) Palm Valley. Lane 9 is the negative control, lane 10 is *N.multiformis*, Lane 11 is *N. europaea* and lane 12, a mixture of the two positive controls.

Nitrifying Potential

TABLE 2. Final pH measurements from rough and sand PNA slurries taken after 5 days of incubation with or without an acetylene headspace. s.d.= standard deviation for n = 3.

		pH		
Soil		+ acetylene (± s.d.)	-acetylene (± s.d.)	delta
Indian Ridge				
	Rough	7.1 (0.2)	5.9 (0.03)	1.2
	Sand	7.8 (0.03)	7.7 (0.1)	0.1
Santa Rosa				
	Rough	7 (0.1)	6.2 (0.5)	0.8
	Sand	7.9 (0.04)	7.6 (0.04)	0.3
Heritage Palms				
	Rough	7.2 (0.1)	6.9 (0.3)	0.3
	Sand	7.5 (0.2)	7.9 (0.04)	0.4
Palm Valley				
	Rough	6.7 (0.1)	5.3 (0.2)	1.4
	Sand	7.0 (0.03)	6.7 (0.1)	1.0

TABLE 3. Potential Nitrification Activity (PNA) measurements for rough and sand PNA slurries incubated for 5 days at 28 º C either with or without an acetylene headspace. s.d.= standard deviation for n = 3.

	PNA [mg NO_3^- Kg^{-1} day^{-1}]		
Soil	+ acetylene	-acetylene	AOB PNA (± s.d.)
Indian Ridge			
Rough	0.4	24	23.6 (± 1.2)
Sand	0	0	0
Santa Rosa			
Rough	1	23	22 (± 4.5)
Sand	0	0	0
Heritage Palms			
Rough	0.6	10	9 (± 7)
Sand	0	4	4 (± 2.4)
Palm Valley			
Rough	1	36	34.7 (± 0.2)
Sand	0	9	9.1 (± 0.7)

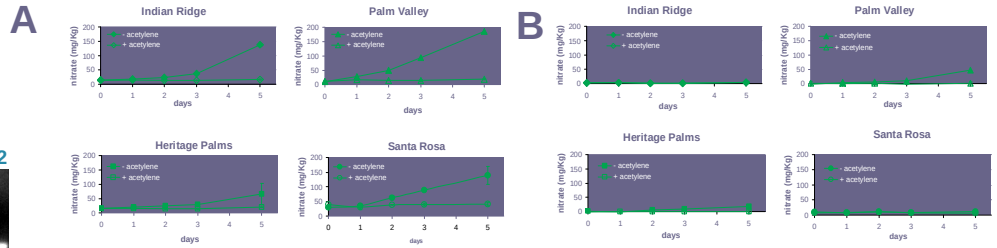

Figure 1. Nitrate accumulation in soil slurries of samples taken from the rough (A) and the sand (B) at each of the four courses. Half of the replicates were treated with a 1 % (v/v) headspace of acetylene. Slurries were conducted in 1.0 mM ammonium and 1.5 mM phosphate buffer on a rotary shaker at 28 º C. Gas chromatography of headspace verified O_2 and acetylene in the headspace (data not shown).

CO_2 Incorporation

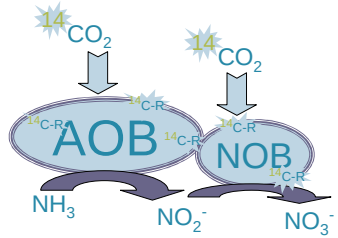

Figure 3. Diagram of carbon sequestration and nitrification by AOB and NOB.

Our Findings

The goal of this study was to examine the effects of nitrogen fertilization and irrigation water sources on activities and community structure of free-living, nitrifying, carbon fixing, microbial communities.

- v Acetylene inhibited nitrification in all soils suggesting autotrophic nitrification at all sites.
- v PNA and delta pH measurements indicate differences in activity between the 4 golf courses.
- v PNA measurements were significantly greater ($P > 0.05$) for the golf course receiving groundwater.
- v PNA measurements were greater for the rough soils than for the sand traps .
- v Diversity measurements suggest a predominance of *Nitrosospira* sps. at all four courses.

Future Directions

- v AOB and NOB abundance measurements should correlate with PNA measurements.
- v Carbon sequestration should track PNA measurements (Figure 3).
- v Carbon incorporation into biomass is anticipated to be greater in the rough than in the sand due to presence of rhizosphere.