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Original article

Nitrification activity and levels of inorganic nitrogen in soils of a semi-arid ecosystem following a drought-induced shrub death

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ABSTRACT

In arid ecosystems, higher rates of biogeochemical cycles occur in soils under shrub canopies than in inter-shrubs spaces. Therefore, changes in shrub cover may have great impact on ecosystem functioning. Recently, a drought-induced massive shrub death was observed in the semi-arid northern Negev Desert in Israel. The aim of this study was to examine the consequences of the shrub death on the levels of soil inorganic nitrogen and the structure of ammonia-oxidizers communities. Compared to soil samples from the winter that preceded the shrubs death, soil samples that were collected at the end of the first winter following the shrub death contained significantly higher nitrate concentrations, exhibited lower ammonia oxidation potential and similar community structure of ammonia oxidizers. In addition, the numbers of ammonia-oxidizers were higher in the soil under dead *Thymelaea hirsute* shrubs than under live shrub canopies. The results suggested that the activities of the nitrogen transforming microbes were moderately affected by the drought and resulted in nitrate accumulation due to the absence of major nitrogen consumers (shrubs). During rainfall events, this nitrate can be washed away by run-off and contaminate downstream water bodies or alternatively denitrified to gaseous nitrogen (including nitrogen oxides).

1. Introduction

Climatic changes are affecting large areas around the globe and are manifested by an increase in annual average temperatures and a decrease in annual average precipitation and the number of precipitation events, along with an increase in precipitation intensity [1]. Such changes can have severe effects on the functioning of terrestrial ecosystems through their impact on the activities and structure of biological communities [2]. Consequently, the services provided by these ecosystems can be impeded [3].

Dry lands are characterized by their negative water balance, with low inputs from precipitation and high losses through evapotranspiration [4] and their ecosystems are considered to be highly susceptible to changes in climate regimes [5]. Desertification processes, such as shifts in an arid ecosystems' vegetation cover, can also be facilitated by climate changes [6]. A phenomenon known as global-change-type drought vegetation die-off, in which large scale mortality of vegetation takes place due to prolonged droughts, was previously reported [7,8]. Such events may trigger a chain of processes that leads to dramatic alterations in ecosystem functioning. Biogeochemical cycles are temporally and spatially limited in arid ecosystems. Precipitation pulses, which appear intermittently during several "wet" months of the year, determine the initiation of organic matter decomposition and nutrient cycling [9,10] while perennial shrubs form spatial "hotspots" where higher rates of biogeochemical activities take place [11,12]. Therefore, alterations in plant cover (death of the shrubs or replacement by invaders) are expected to affect the rates of biogeochemical cycles, such as the carbon and the nitrogen cycles [13,14].

In arid ecosystems, nitrogen availability constitutes the second most limiting factor (after water) in determining ecosystem productivity [15,16]. Nitrification (oxidation of ammonia to nitrate via nitrite) has a key role in determining nitrogen bioavailability. Its product, nitrate, is readily removed from the ecosystem by reduction to gaseous nitrogen or through leaching after intense rain events [9,17]. Indeed, significant amounts of nitrogen (nitrate) have been detected in the sub-soil of many deserts, possibly due to changes in plant cover that occurred at the onset of the arid climate in these regions some 10,000 years ago [18]. In line with this,

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a previous study [19] has demonstrated that one of the major differences in soil chemical properties between arid and semi-arid regions is the accumulation of nitrate in the former. Recently, the same semi-arid region [19] was exposed to a prolonged drought period that caused a massive shrub death [20]. Following this increase in the aridity level and the dramatic changes in plant cover, we hypothesized that these changes will be manifested in soil chemical properties and soil microbial activities. The objectives of this study, therefore, were to look at the soil chemical properties and, in particular, the levels of inorganic nitrogen species in the affected semi-arid region of the northern Negev Desert. In addition, the diversity and abundance of the ammonia oxidizing microbes were determined since ammonia oxidizers catalyze the first, and what is generally considered the rate-limiting step of nitrification.

2. Materials and methods

2.1. Research location and soil sampling

Soil samples were collected from Sayeret Shaked Park, a long-term ecological research site (LTER). This is an Israeli-LTER located in the semi-arid zone in the northern Negev Desert (31°17′N, 34°37′E). Average daily minimum winter temperatures are 6–8 °C (November–March), and average daily maximum summer temperatures are 32–34 °C (May–September) [21]. A massive death event of the dominant shrubs in this site was observed in the summer of 2008. Soil samples were collected in March 2008 (the winter that preceded the shrub death, the collection was part of an ongoing project in this region), in April 2009 (the winter after the shrub death, which was an extremely dry winter) and in March 2010. Soil samples (100–200 g) were taken from beneath the canopy (0–10 cm depth) of three to five live *Noaea mucronata* (*Nm*) plants, three to five live *Thymelaea hirsute* (*Th*) plants, and from four to five inter-shrub patches (ISP). In the winter of 2009, additional samples were taken from sites where dead *N. mucronata* (*Nm-dead*) plants and dead *T. hirsute* (*Th-dead*) were located-three samples of each. Samples were collected in polyethylene bags, brought to the lab within 90 min of collection, and were immediately prepared for analyses as described below.

2.2. Soil chemical analysis

Soil samples were sieved (<2.0 mm) and dried at 65 °C for 24 h. Nitrogen species were extracted from 15 g soil in 60 ml KCl (1 M) by shaking the samples for 60 min at room temperature and filtering them through a 0.45 μm filter. Total ammonia nitrogen (TAN) was determined by the Nessler method, nitrite by the sulfanilamide method [22] and nitrate by the 2nd derivative method [23]. Water content was determined after drying overnight at 105 °C, pH and conductivity (EC; mS/cm) were measured using a TDS/conductivity meter in a 1:1 (W/V) mixture of soil and distilled water (15 g per 15 ml), and total organic matter (OM) was determined by combustion of the 105 °C dried soil samples at 450 °C.

2.3. Ammonia oxidation potential (AOP)

AOP was determined by incubating 10 g of fresh soil in a medium (60 ml) containing 25 mM K_2HPO_4 (pH 7.8), 0.5 mM $(NH_4)_2SO_4$ and 10 mM $KClO_3$ for 6 h at 25 °C and continuous shaking (200 rpm). $KClO_3$ was included in order to inhibit nitrite oxidizers [24]. The slurries were centrifuged for 10 min at 10,000 rpm and the supernatant was filtered through 0.45-μm GF-3 filter papers. Nitrite accumulation was determined as above. Nitrite levels at time zero were determined for all samples and subtracted from the values obtained after 6 h.

2.4. DNA extraction and PCR amplifications

Genomic DNA was extracted from 0.5 g soil, with the PowerSoil™ DNA Isolation Kit (MO BIO Lab. Inc., Solana Beach, CA), and quantified using NanoDrop (ND-1000, NanoDrop Inc., Wilmington, DE). The primers used for each application and the amplification conditions are summarized in Table 1. The AOB 16S rRNA gene fragments for the Denaturing Gradient Gel Electrophoresis (DGGE) analysis were amplified by nested PCR strategy. First, a larger DNA fragment was amplified by the β-AMO primers, followed by a second PCR reaction using the CTO primers (Table 1). This approach yields more DNA bands by the DGGE analysis. PCR reactions (50 μl) contained approximately 20 ng DNA, 2.5 mM $MgCl_2$, 250 μM of each of the four dNTPs, 25 pmol of each of the primers, 50 μg BSA, and 1.5 U of REDTaq DNA polymerase (Sigma, St. Louis, MO). Amplifications were carried out in a TGradient thermocycler (Biometra, Gottingen, Germany).

DGGE analysis was performed with a Dcode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA) under the following conditions: 1-mm thick 8% polyacrylamide gel, with a denaturing gradient of 15–50% and 35–50% urea–formamide for the archaeal *amoA* gene fragments and the bacterial 16S rRNA gene fragments, respectively (Table 1). The 100% denaturant is a mixture of 7 M urea and 40% deionized formamide. PCR products were run at 60 °C, for 1160 min at 80 V and 980 min at 70 V for archaeal and bacterial PCR-amplified DNA fragments, respectively. Polyacrylamide gels and all DGGE solutions were prepared according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). Ethidium bromide-stained gels were visualized on a Gel Doc XR gel imaging system (Bio-Rad, Hercules, CA, USA).

2.5. Real time PCR of amoA genes

The abundance of AOA (ammonia oxidizing archaea) and AOB (ammonia oxidizing bacteria) was estimated by enumerating the putative archaeal *amoA* and the bacterial *amoA* genes, respectively.

Table 1
PCR primers and conditions applied in this study.

Target gene	Application	Primers	Conditions	Size (bp)	Reference
AOA-*amoA*	DGGE and qPCR	Arch amoAF-5′-ttatggtctggcttagacg-3′ Arch amoAR-5′-gcggccatccatctgtatgt-3′	95 °C-15 min, then 35 cycles of: 95 °C-35 s, 54 °C-45 s, 72 °C-50 s	635	[55]
AOB-*amoA*	qPCR	amoAF-5′-gggghttytactggtggt-3′ amoA2R-5′-cccctckgsaaagcctt cttc-3′	The same as for the Arch primers	491	[56]
AOB-16S rRNA	DGGE primary reaction of nested PCR	β-AMO141F-5′-tggggrataacgcaycgaaag-3′ β-AMO1320R-5′-agactccgatccggactacg-3′	94 °C-5 min, then 30 cycles of: 94 °C-30 s, 55 °C-30 s, 72 °C-80 s, final elongation step-10 min at 72 °C	1179	[57]
AOB-16S rRNA	DGGE secondary reaction of nested PCR	CTO189F-5′-(GC clamp)-gagraaagcagg ggatcg-3′ CTO649R-5′ctagcyttgtagtttcaaacgc-3′	The same as for the β-AMO primers	460	[58]