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Comparative study of nitrogenase activity in different types of biological soil crusts in the Gurbantunggut Desert, Northwestern China

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ABSTRACT

Biological soil crusts cover large areas of the Gurbantunggut Desert in northwestern China where they make a significant contribution to soil stability and fertility. The aim of this study was to quantify the potential nitrogen-fixing activity (NA) of different types of biological soil crusts in the Gurbantunggut Desert. The results suggest that NA (nmol $C_2H_4\,m^{-2}\,h^{-1}$) for each type of crusts was highly variable. Seasonal variation was also important, with all three types of crusts responding in a similar way to changes in environmental conditions. From March to May, NA was relatively low for all crust types. During this season, NA was 2.26×10^3 for cyanobacterial crust followed by lichen crust (6.54×10^2) and moss crust (6.38×10^2). From June to October, all crust types reached their highest level of NA, especially lichen crust and moss crust ($p < 0.01$). The NA of cyanobacterial crust (9.81×10^3) was higher than that of lichen crust (9.06×10^3) and moss crust (2.03×10^3). From November to February, when temperatures were consistently low (<0 °C), NA was at its lowest level, especially in cyanobacterial crust (4.18×10^2) and moss crust (5.43×10^2) ($p < 0.01$). Our results indicate that species composition is critical when estimating N inputs in desert ecosystems. In addition, all three types of crusts generally responded in a similar way to environmental conditions. The presence of N fixation activity in all crusts may contribute to the maintenance of fertility in sparsely vegetated areas and provide surrounding vascular plant with fixed nitrogen.

1. Introduction

Net primary productivity in terrestrial ecosystems is often limited by nitrogen availability (Vitousek and Howarth, 1991). During desertification of semi-arid grasslands, this limitation may intensify (Dregne, 1983), but relatively little is known about the factors affecting the nitrogen status of desert soils. Nitrogen inputs in arid lands result largely from N_2 fixation (Belnap, 2002b; Evans and Ehleringer, 1993; Rychert et al., 1978; Skujins, 1984) by biological soil crusts and free-living, heterotrophic bacteria (MacGregor and Johnson, 1971; Zaady et al., 1998). In desert ecosystems, nitrogen fixed by the heterocystic cyanobacteria (*Anabaena*, *Calothrix*, *Cylindrospermum*, *Dicothrix*, *Hapalosiphon*, *Nodularia*, *Nostoc*, *Plectonema*, *Schizothrix* and *Scytonema*) (Harper and Marble, 1988), non-heterocystous cyanobacteria (*Lyngbya*, *Microcoleus*, *Oscillatoria*, *Phoridium* and *Tolypothrix*) (Belnap, 1996; Rogers and Gallon, 1988) and cyanolichens (*Collema* spp. and *Peltula* spp.) that occur in the biological soil crusts can be the major source of nitrogen (Evans and Ehleringer, 1993). This is especially true for regions where rainfall and inputs of N resulting from human activities are low. Cyanobacteria can also live epiphytically on soil mosses and lichens that have green algae as phycobionts; thus this consortium of organisms can also show N fixation activity (Peters et al., 1986).

Estimations of N_2 fixation by soil crusts in arid and semi-arid areas vary widely (Aranibar et al., 2003; Belnap, 2002b; Hartley and Schlesinger, 2002; Zaady et al., 1998). It is difficult to compare values which can be reported using different units of time, for example from hourly to annual rates, and at different spatial scales, for example from square centimeters to hectares (Aranibar et al., 2003). Although the importance of N_2 fixation in soil crusts has been widely studied in many deserts throughout the world, this has not been the case in China. Until now, most studies of nitrogenase activity in deserts in China were focused on legumes (Ci and Gao, 2005; Shen and Jing, 2003), and few mentioned the possible role of biological soil crusts in N_2 fixation (Li et al., 2001; Zhang et al., 2005).

The Gurbantunggut Desert (44°11′–46°20′N, 84°31′–90°00′E) is the largest fixed and semi-fixed desert in China with an area of 48,800 km^2. In recent years there has been a significant increase in

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utilization, in particular for livestock grazing, agricultural production and energy exploration. Soil surface disturbances associated with these uses have been repeatedly shown to convert species-rich biological soil crusts dominated by late successional lichens and mosses, to species-poor crusts dominated by early successional cyanobacteria. The recovery of disturbed biological soil crusts is extremely slow. In order to protect biological soil crusts in this desert, a number of projects have been undertaken to study their role in soil stabilization, increasing the fertility of soil, minimizing wind and water erosion and adding to soil organic matter (Zhang, 2005; Zhang et al., 2006). The purpose of this study was to quantify the nitrogen-fixing activity in different types of soil crusts in the Gurbantunggut Desert using acetylene reduction assays (ARA) and to discuss the factors influencing NA.

2. Materials and methods

2.1. Study area

The study was conducted in the Gurbantunggut Desert, which is situated in the center of the Jungger Basin, in the Xinjiang Uygur Autonomous Region of China. The Himalayan Range to the south produces a 'blocking effect', preventing moist air currents from the Indian Ocean reaching the area, and resulting in the vast expanse of arid terrain. Mean annual rainfall is approximately 79.5 mm, falling predominantly during spring. Mean annual evaporation is 2606.6 mm. The average annual temperature is 7.26 °C. Wind speeds are greatest during late spring, averaging 11.17 m s^{-1}, and are predominantly from the WNW, NW and N directions (Zhang et al., 2007). Natural vegetation in the area is dominated by *Haloxylon ammodendron* and *Haloxylon persicum* (Chenopodiaceae), with a vegetation cover of less than 30%. The land is characterized by massive, dense, semi-fixed sand dunes with stable moisture content and covered by biological soil crusts consisting of various combinations of bacteria, cyanobacteria, algae, mosses and lichens. Scattered shrubs can be associated with the soil crusts. These biological soil crusts grow favorably during cool, wet, periods, in fall and early spring, utilizing not only rainfall, but also dew and fog (Du, 1990; Kidron et al., 2002; Zhang et al., 2002). The study was conducted in the southern part of the Gurbantunggut Desert which contains biological soil crusts typical of those (algae-dominated crust, lichen-dominated crust and moss-dominated crust) found throughout the desert (Zhang et al., 2002, 2004) (Fig. 1).

2.2. Field experiment layout

In 2003, a typical, longitudinal sand dune (44°32′30″N, 88°6′42″E) was selected as a permanent site. We selected an area covered only in biological soil crusts in order to avoid any complications from the presence of vascular plants. From the interdune area, we randomly selected ten 50 cm × 50 cm plots at approximately 10 m intervals. The thickness and cover of biological soil crust were recorded for each plot. Three types of soil crusts were collected from each plot between March 2005 and February 2006: (1) cyanobacterial crust which is heavily dominated by the cyanobacterium *Microcoleus vaginatus*. Sand particles are bound tightly not only by the fine filaments of this cyanobacterium but also by the mucilage it produces, thus protecting the sand surface from wind and water erosion; (2) lichen crust, which included *Collema tenex*, *Psora decipiens*, *Xanthoria elegans*, *Acarospora strigata*, and *Lecanora argopholis*. Lichen-dominated soil crust is the predominant type of biological soil crusts in the desert where different species of lichen can be black, white, brown, yellow or blue in color; (3) moss crust, which is mainly dominated by *Tortula desertorum* and *Bryum argenteum*.

Crust samples were collected by inserting a 5.4-cm diameter aluminium container into the soil crust, then carefully removing it to provide a sample of crust with a surface area of 22.89 cm^2 and approximately 2 cm deep. Ten replicates from each type of crust were collected, making a total of 30 samples for each sampling period. These were transported without delay to the laboratory of the Xinjiang Institute of Ecology and Geography, Chinese Academy of Science in Urumqi.

2.3. Acetylene reduction activity

In the laboratory, the samples were analyzed immediately for nitrogenase activity (NA). NA was estimated using the acetylene reduction assay (ARA), based on the ability of the nitrogenase enzyme to catalyze the reduction of N_2 to ammonium (nitrogen fixation), as well as the reduction of acetylene to ethylene (Aranibar et al., 2003). Samples were placed in clear, 60-ml gas-tight tubes and the entire crust surface was equally and completely moistened with distilled water. Tubes were injected with enough C_2H_2 to create a 10% C_2H_2 atmosphere. After injection, samples were incubated for 24 h at 26 °C. Sub-samples (0.5 ml) of the head space within the tubes were analyzed for C_2H_2 and ethylene (C_2H_4) content on a Trace GC 2000 gas chromatograph, fitted with flame ionization detector, and Al_2O_3/S column, using N_2 as the carrier gas (1.5 ml/min). Results of the observed NA were expressed as ethylene production (nmol C_2H_4 m^{-2} h^{-1}) per unit time on a surface area basis (Belnap, 2002b).

Converting C_2H_4 values is controversial, as the ratio used varies widely depending on the organism and habit conditions. The ratio of ethylene produced to N fixed varies widely for different soils, microbial communities and environmental conditions. In Sweden, conversion factors ranged from 1 to 15.7 for different soils and water contents (Nohrstedt, 1983), and in the high Arctic from 0.022 to 4 for different microbial communities (Liengen, 1999). However, existing literature commonly reports N_2 fixation applying the theoretical 3:1 ratio (Ischiei, 1980; Rychert and Skujins, 1974; Skarpe and Henriksson, 1986). For the purpose of comparison, this ratio was also used in this study to describe the nitrogen fixation activity associated with biological crusts.

2.4. Statistical analyses

All statistical analysis was done using an SPSS statistical package. ANOVA procedure was used to understand how crust types and sampling time affect NA. Tests of between-subjects effects showed the results more clearly. Results are reported as significant when $p < 0.01$ unless otherwise noted.

3. Results and discussion

3.1. The potential NA of different types of biological soil crusts

Rates of ethylene production (as indications of nitrogenase activity and N_2 fixation) are presented in Fig. 2. The results suggest that potential nitrogenase activity (NA) for each type of crust varied considerably depending on the season.

From March to May, rates of ethylene production were quite low for all crust types, but of these, the cyanobacterial crust reached the highest rate of ethylene production (2.26×10^3 nmol C_2H_4 m^{-2} h^{-1}), followed by lichen crust (6.54×10^2 nmol C_2H_4 m^{-2} h^{-1}). Moss crust produced the lowest rate (6.38×10^2 nmol C_2H_4 m^{-2} h^{-1}).

From June to October, each type of crust reached its highest rate of NA, particularly in the case of lichen crust and moss crust ($p < 0.01$). Ethylene production rates of cyanobacterial crust (9.81×10^3 nmol C_2H_4 m^{-2} h^{-1}) and lichen crust (9.06×10^3 nmol