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Study of soil cyanobacteria along a rural-urban gradient

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ABSTRACT

Urbanization is one of the modern and critical factors adversely affecting global biodiversity. The impact of urbanization on soil microbial diversity has sporadically been studied of cyanobacterial diversity. The present study analyzes morphological as well as molecular diversity of cyanobacteria along a rural-urban gradient using a culture based approach. In total, 22 cyanobacterial morphotypes (14 genera and 21 species) from five orders were reported; dominated by the members of the order Nostocales. In general, cyanobacterial diversity decreased from rural to urban areas; with N_2-fixing heterocystous forms dominating the cyanobacterial flora of the urban area. The values of Shannon–Weaver (2.56) and Simpson's (2.32) indices suggested higher cyanobacterial diversity in the rural area compared to that of the sub-urban and urban areas. Statistical analyses established the importance of physico-chemical factors in structuring the cyanobcaterial communities along the gradient with soil characteristics such as – pH, organic carbon, nitrogen and bulk density, directly as well as indirectly.

1. Introduction

The Millennium Ecosystem Assessment report [1] suggests that during the past 60 years, land cover change has resulted in severe global terrestrial biodiversity loss. Among various factors, advancing urbanization has been responsible for extensive modifications of the landscape. Urbanization alters the quality of air, water and soil, temperature regime and rainfall patterns of the environments, which could lead to the destruction and fragmentation of the natural habitats and protuberances in biological communities [2–4], thereby, possing a major threat to the biodiversity. McDonnell et al. [5] proposed the concept of rural-urban-gradient to model the ecological effects of urbanization on soil-biological systems. Besides variations in several physico-chemical parameters, such gradients differ in the amount of built-up area (buildings, roads and asphalt covered paths) and differences in the habitat maintenance operations. The length of the selected gradient/transect vary depending upon the kind of organism/group is to be studied. A greater length of transect is required for the study of macrophytes compared to their microbial counterparts, with basic rock composition remaining similar along the gradient.

Due to their high diversity and abundance, microorganisms constitute a bulk of soil genetic resource [6,7]. Soil microbes play a crucial role in the functioning of soil ecosystems directly through cycling of soil nutrients, decomposition of soil organic matter, detoxification of pollutants, and indirectly as a crucial regulator of global climate. However, soil microbial population and associated ecosystems services are sensitive to changes in external conditions imposed by land use modifications and human management practices (i.e., urbanization, agricultural intensification and industrialization, etc.) [7–9]. Studies suggest that urbanization has profoundly impacted the function and composition of soil microbial communities [10–12]. The resulting change could be used for evaluating the soil and ecosystem function. However, due to methodological limitations and site specificity, often factors affecting the structure and functioning of soil microbial communities (i.e., abundance, spatial organization, etc.) are not clearly understood [13], which limits our understanding of the role of soil microbial diversity in ecosystem services [14]. Information is mainly available on the diversity of plants, arthropods, fungi, bacteria, along the urbanization gradient.

Cyanobacteria (also known as Blue-green algae), is a morphologically diverse group of photooxybacteria, with worldwide distribution in diverse ecosystems. They represent one of the major eubacterial groups and possess unique characteristics of oxygenic photosynthesis and N_2-fixation among the prokaryotes. In soil, they provide fixed nitrogen and photosynthetically fixed carbon to plants and other soil components [15,16]. In addition, they play an important role in ecosystem function, such as stabilization of soil or mobilization of sand surfaces by excretion of extracellular polysaccharides, hinder wind erosion and facilitate the initial establishment of higher plants and subsequent feedback mechanisms [17–20]. In recent times, they have emerged as a valuable

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source of biologically active natural products [21].

Apart from their ecological significance, an understanding of the diversity and species composition of cyanobacteria along an urban-rural gradient (increasing disturbance) is crucial from an agronomic viewpoint also. As rural and sub-urban areas, located at the periphery of urban settlements, act as a crucial supply chain of agricultural products to the cities.

The present study is one of the first to explore the impact of urbanization on soil cyanobacterial community that to a large extent remain unexplored. It focuses on the follow-up changes of cyanobacteria diversity and distribution along a rural-urban gradient with exposure to the associated physico-chemical soil properties as an outcome of this anthropogenic phenomenon. The findings could further be employed to characterize the cyanobacteria and other soil microbes from areas under severe human-induced environmental modifications [22].

2. Materials and methods

2.1. Study site and sampling

Soil samples were collected along a rural-urban gradient in Varanasi, India which is located at a latitude of 25°19′14·86 N and longitude of 82°58′12·30 E. Three agro climatic areas (urban, sub-urban and rural) were selected along the gradient. The distance between the sampling areas (rural, sub-urban and urban) was 1–3 km. In addition to differences in land cover, the gradient differed in habitat maintenance operations. ArcView GIS program 10.3 was used to map the sampling site. Samples were collected from June to September 2015 (favourable season for cyanobacterial growth), at 15 day intervals. Soil samples were collected from various points distanced 100 m apart to each other along the transect. At each soil collection site, a permanent sampling plot approximately 2 × 2 m was marked for periodic collections. From each sampling site, three cores were collected up to a depth of 15–20 cm (form the centre as well from the corners) using a core sampler fitted with butyrate sampling tube of 2.5 cm diameter. The built-up area in rural, sub-urban and urban was zero (no buildings), 30 and 60%, respectively. Collected samples were stored at 4 °C for further processing. Soil samples collected from a particular site were mixed and homogenized to produce a representative composite soil sample. Samples were air dried and sieved (2 mm) prior to their physico-chemical analyses.

2.2. Soil characterization

The electrical conductivity (EC) and pH of collected samples were determined according to Sparks [23], soil texture (clay, silt and sand) as per [24], and bulk density by the core sampler method [25]. Samples were oven dried to measure soil moisture content. Organic carbon (OC) was measured following the chromic acid digestion method [26]. Micronutrients, such as Fe, Cu, Zn and Mn, were extracted in diethylene triamine penta-acetic acid (DTPA) and quantified as described by Lindsay and Norwell [27]. The available nitrogen (N) was measured by the method developed by Subbiah and Asija [28], phosphorus (P) content by Olsen et al. [29] and potassium (K) by Hanway and Heidal [30]. Analysis of soil microbial biomass was carried out following the method by Brookes et al. [31,32] and Vance et al. [33].

2.3. Isolation and identification of cyanobacteria

Cyanobacteria were isolated from the composite soil samples using serial dilution agar spread plate method. BG-11 [34], Chu-10 [35] and Allen-Arnon [36] growth media, with and without nitrogen were used for the isolation of cyanobacteria. Cultures were incubated in a culture room, at 28 ± 1 °C and illuminated with a combination of white and day light fluorescent lamps giving an average light intensity of 70 μmol $m^{-2} s^{-1}$ with a 14:10 h light-dark rhythm. After 2–3 weeks, single-isolated colonies from agar plates were picked up and sub-cultured in their respective liquid growth medium. Cyanobacterial isolates were identified on the basis of their cell and filament morphology according to [37–43].

Table 1
Mean (± SD) soil properties along the urbanization gradient.

Soil properties	Urban	Sub-urban	Rural
pH	7.7 ± 0.17^{a}	7.3 ± 0.16^{a}	6.7 ± 0.20^{b}
EC (μs cm^{-1})	202.5 ± 7.5^{a}	231.7 ± 13.5^{ab}	271.4 ± 9.2^{b}
Clay (%)	12.4 ± 0.02^{a}	13.1 ± 0.12^{ab}	13.3 ± 0.12^{a}
Silt (%)	28.8 ± 0.53^{a}	27.5 ± 0.25^{ab}	26.3 ± 0.64^{b}
Sand (%)	56.0 ± 0.43^{a}	58.3 ± 0.27^{a}	55.0 ± 0.54^{a}
BD (Mg m^{-3})	1.39 ± 0.01^{a}	1.36 ± 0.02^{a}	1.31 ± 0.01^{b}
Moisture (%)	6.2 ± 0.12^{a}	6.7 ± 0.26^{ab}	7.2 ± 0.08^{b}
MBC (μg g^{-1})	107.4 ± 1.2^{a}	121.3 ± 4.7^{b}	134.2 ± 3.7^{b}
MBN (μg g^{-1})	10.2 ± 0.16^{a}	11.5 ± 0.40^{b}	12.5 ± 0.32^{b}
MBP (μg g^{-1})	5.1 ± 0.15^{a}	5.8 ± 0.16^{b}	6.3 ± 0.22^{ab}
OC (%)	0.36 ± 0.02^{a}	0.42 ± 0.02^{b}	0.44 ± 0.01^{b}
N (kg ha^{-1})	133.0 ± 6.1^{a}	149.9 ± 8.3^{ab}	182.3 ± 4.7^{b}
P (kg ha^{-1})	15.1 ± 1.2^{a}	18.6 ± 0.87^{b}	20.1 ± 0.58^{b}
K (kg ha^{-1})	142.9 ± 3.7^{a}	154.7 ± 3.7^{ab}	170.8 ± 5.2^{b}
Fe (mg kg^{-1})	6.4 ± 0.24^{a}	7.5 ± 0.32^{ab}	8.6 ± 0.51^{b}
Cu (mg kg^{-1})	1.1 ± 0.06^{a}	1.5 ± 0.14^{a}	1.7 ± 0.06^{a}
Zn (mg kg^{-1})	0.3 ± 0.04^{a}	0.4 ± 0.02^{a}	0.5 ± 0.02^{a}
Mn (mg kg^{-1})	3.9 ± 0.39^{a}	5.2 ± 0.22^{b}	5.8 ± 0.18^{b}

Different letters indicate significant differences ($P < 0.05$ by Tukey's HSD test) between the sites.

2.4. Genomic DNA extraction, PCRs and 16s rDNA sequences analysis

Genomic DNA was extracted using QIAamp DNA minikit (Qiagen, Hilden, Germany), according to the manufacturer's established protocol. The taxonomic identities of the morphotypes were determined by sequencing the conserved 16S rDNA gene. The 16 S rDNA gene was amplified with the Primers CYA781R (5′-AGA GTT TGA TCC TGG CTC AG-3′) and CYA361F (5′-AAG GAG GTG ATC CAG CCG CA-3′) [44]. The 50 μl reaction mixture contained 50 ng of template DNA, 10× PCR buffer, 2.5 mM dNTPs, 1 U Taq DNA polymerase and 10 pM of each primer pA and pH [45]. A negative control (PCR mixture without DNA template) was included for each PCR reaction. The amplification was carried out in a thermal cycler (MJ Mini™, PTC-1148, Bio-Rad, Singapore) under the following amplification conditions: initial denaturation of 5 min at 94 °C, followed by 40 cycles of 40 s at 94 °C, 40 s at 53 °C, and 1 min 30 s at 72 °C and a final extension period of 10 min at 72 °C. PCR products were sent to SCI Genome Chennai, India, for sequencing. DNA sequences were refined and compared with basic sequence alignment with the database in the National Center for Biotechnology Information Blast (www.ncbi.nlm.nih.gov/BLAST). All the sequences were aligned by multiple sequence alignment program clustal W [46]. The phylogenetic tree was constructed using the MEGA6 software [47] using neighbour-joining method. The tree reliability was evaluated by bootstrap analysis with 1000 replications using elimination of gaps/missing data with a site coverage cut off of 95%. Phylogenetic tree evolutionary distances were computed using the Kimura 2-parameter model [48]. The sequences obtained were deposited in Gen-Bank nucleotide-sequence database under the accession numbers KY129695 and KY129716.

2.5. Cyanobacterial diversity indices

Cyanobacteria diversity along the rural-urban gradient was calculated by the Shannon–Weaver index of diversity (H_m) [49] and Simpson's index of dominance (D) [50], for both richness and proportion of individuals [51]. The diversity and dominance indices were calculated using the following formulas [52]:

A. Shannon–Weaver index of diversity (H_m):