Rhizosphere ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Soil enzymes and microbial endophytes as indicators of climate variation along an altitudinal gradient with respect to wheat rhizosphere under mountain ecosystem

Rahul Jain, Anita Pandey *

Biotechnological Applications, G. B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora, 263643 Uttarakhand, India

ARTICLE INFO

Article history:
Received 9 May 2016
Accepted 27 July 2016

Keywords:
Wheat rhizosphere
Climate
Soil enzymes
Endophytes
Indian Himalayan Region

ABSTRACT

Microorganisms are always a crucial part of any ecosystem as they carry out various activities to maintain the sustainability of that ecosystem. Microbial activity in soils depends on the chemical structure of soils and the climatic conditions. In mountain ecosystem, change in climate is observed along with the change in altitude. Such change in climate, in turn, are likely to cause change in the community structure of microorganisms. In the present study, enzyme activities in soils and root colonization with respect to wheat rhizosphere are studied at three different altitudes (referred as S1, S2, S3 with increasing altitude) for two consecutive years. Activity of six soil enzymes including acid and alkaline phosphatase, β-glucosidase, aryl sulfatase, urease and dehydrogenase are estimated. All the soil enzymes, except dehydrogenase, were found to be governed by the soil quality defined by its nutrient content in the respective soil. Dehydrogenase enzyme activity was found to increase with the altitude indicating high rate of biological oxidation of soil organic matter at high altitude (S3) by native microbiota. Fungal counts in soil at higher altitude (S3) in both bulk soil as well as rhizosphere were minimum. Wheat plant roots at each altitude were observed to be colonized with specific type of microbial structures; S1 with higher population of small vesicular and bacterial endophytes, S2 possessing large vesicles and dark septate endophytes, and S3 showing lobed vesicles and dark septate endophytic structures. Total root colonization, recorded in both the study years, was maximum in the roots of S3 site followed by S2 and S1. Such patterned variations, arising due to altitudinal difference, may be considered as indicators for climate manipulations at small scale. The study will have implications in understanding the role of soil enzymes and the microbial communities, endophytes in particular, in wheat productivity grown in high altitudes under mountain ecosystem.

1. Introduction

Extracellular enzymes in soil are released mainly due to the microbial activities leading to various ecological functions. It includes nutrient recycling in the soil by degradation of soil organic matter (SOM) to maintain the soil health. Microbial components, including mycorrhizae present in the roots, are the key producers of soil enzymes (Ladd, 1978). However, plant roots are also responsible for release of some enzymes in the form of root exudates. Microorganisms are the source for various enzymes for the uptake of a variety of nutrients required for their metabolic activities. Therefore, the enzyme activities in soil depend on the chemical composition of soils including SOM and its carbon and nitrogen content (Schnecker et al., 2014; Sinsabaugh et al., 2008). Overall, the enzyme activity in soil acts as the sensor of soil microbial and nutrient status and its fertility. The most widely studied enzymes include hydrolytic enzymes which mainly cause decomposition of cellulose and lignin (Allison et al., 2007). Extracellular phosphatases are important in mineralization of phosphorus from nucleic acids, phospholipids, etc. (Turner et al., 2002). Similarly, aryl sulfatase and urease are responsible for decomposition of sulphur and nitrogen compounds, respectively; dehydrogenase plays role in the soil respiration through biological oxidation of SOM (Wolińska and Stępniewska, 2012).

Microbial diversity and their populations are the important factors that govern the enzyme activities in soil. The structure of microbial community itself depends on several ecological, both biotic and abiotic, factors such as plant communities, soil structure, soil organic matter, soil pH and moisture, etc. (Li and Chen,

* Corresponding author.
E-mail addresses: anita@gbpihed.nic.in, anitapandey333@gmail.com (A. Pandey).

http://dx.doi.org/10.1016/j.rhisph.2016.07.007
2452-2198/

2004). Climatic variation at micro-scale, for example variation due to altitudinal difference, also affects the microbial communities. This is due to change in climatic factors such as temperature, net precipitation, O_2 availability, etc. These changes enable colonization of higher population of microbes which are adapted to particular type of environments causing reduction in the diversity. The respective change in microbial community leads to specialized microbial processes depending on the climatic conditions.

Indian Himalayan Region (IHR) under mountain ecosystem is diverse in all forms of life including microbial diversity. These mountains support growth of various agricultural crops, forest species and medicinal plants due to varying soil types. The soil structure varies in these hills with respect to the altitudinal gradient and also the climatic conditions. These multiple factors, leading to change in microbial communities, influence the soil enzymatic activities. With an increasing demand of reproducible soil quality indicators based on soil microbiota (Anderson, 2003), there is also great need for identifying the microbial indicators to assess climate variability especially in the mountains which are being affected by climate change. Also, microbial communities in varying climatic conditions from diverse ecosystems, including mountains, are increasingly getting attention in view of their ecological as well as biotechnological relevance (Ciccazzo et al., 2015; Dhakar et al., 2014; Ghildiyal and Pandey, 2008; Massaccesi et al., 2015; Yarzábal, 2014).

The variation, due to changing environmental conditions such as elevation gradient, in case of plant and animals, has been studied in details but pattern for microorganisms including mycorrhiza, remain still poorly understood especially in context of agricultural crops. Wheat (*Triticum aestivum* L.) is a staple crop of India and prescribed varieties are grown in tropical to temperate locations including colder regions of mountain ecosystem under IHR. The present study aims to assess variation in the soil enzymatic activities and microbial colonization along an altitudinal gradient, at three altitudes with respect to bulk and wheat rhizosphere soil in IHR.

2. Experimental

2.1. Site description

The present study was conducted on the farmers' fields at three altitudes referred as S1 (Kosi-Katarmal) in District Almora and S2 (Kalimati) and S3 (Lata) in District Chamoli of the state Uttarakhand under IHR (Table 1, Fig. 1). These sites represent three sets of climatic conditions with respect to cultivation of local wheat. The three sites experience seasonal precipitation as well as snowfalls, variably; S1 with occasional snowfall (once in few years) but no snow cover, S2 with 1–3 days snow cover whereas S3 remains covered by the snow for 15 to 30 days every year. The average temperature and rainfall data of three sites are presented in Table 2.

Table 1
Geographical location of the three study sites in Uttarakhand Himalaya.

S. no.	Site	Altitude (asl)	Latitude	Longitude
1	Kosi-Katarmal, Distt. Almora **(S1)**	1345 m	**N** 29°38′06″	**E** 79°36′36″
2	Kalimati, Distt.Chamoli **(S2)**	1900 m	**N** 30°05′18″	**E** 79°17′09″
3	Lata,Distt. Chamoli **(S3)**	2400 m	**N** 30°29′36″	**E** 79°42′45″

2.2. Soil sampling and plant root collection

Soil sampling was done with respect to the local wheat growing seasons at three different sites. Bulk soil samples from 5–15 cm depth were collected during 3 seasons i.e. before sowing (Initial), during flowering (Mid-N) and after harvesting (Final) from each site, in replicates, while rhizosphere soil samples were collected carefully during flowering season (Mid-R) only. Five samples per site (five replicates) were collected and mixed in laboratory to make composite samples. The soils were kept at 4 °C until further analysis for soil enzymes. Remaining amount of soil was dried at room temperature for chemical analysis after sieving through 2 mm sieve. Root samples were also collected during flowering season from each altitude to find out the total root colonization. All the study parameters were conducted during winters in two consecutive years from 2012–2014.

2.3. Physico-chemical analysis of soil

The soils were analysed for various physical as well as chemical parameters. Soil pH (H_2O) was measured in 1:2 soil/water slurry using a glass electrode and % moisture of fresh soil was measured after drying the fresh soil and percent difference in weight of fresh and dry soil was recorded. Total organic carbon content of soil was measured by chromic acid wet oxidation method as described by Walkley and Black (1934). Total nitrogen was estimated by the method of Parkinson and Allen (1975). Available potassium in the soil samples was determined by Jackson (1958) method using flame photometer. Available phosphorus for acidic and alkaline soils was assessed by the methods described by Bray and Kurtz (1945) and Olsen et al. (1954), respectively.

2.4. Soil enzymes analysis

Six soil enzymes *i.e.* acid phosphatase, alkaline phosphatase, β-glucosidase, aryl sulfatase, urease, and dehydrogenase, were considered in the present study. The selection criteria of these enzymes is based on their importance in soil metabolism and nutrient cycling. Following methods were used for the estimation of these enzymes from soil: 4-nitrophenyl (*p*NP) linked substrate were used for the estimation of acid and alkaline phosphatase (Tabatabai and Bremner, 1969), β-glucosidase (Dick et al., 1996) and aryl sulfatase activity (modified Tabatabai and Bremner, 1970) which are 4-nitrophenyl phosphate, 4-nitrophenyl β-D glucopyranoside and 4-nitrophenyl sulphate (all from Sigma), respectively, in modified universal buffer (Skujins, 1973). Acid phosphatase, β-glucosidase and aryl sulfatase were assayed at pH 5.8 while alkaline phosphatase was assayed at pH 9.5. Briefly, 1 g of soil was taken in 4 ml of buffer of desired pH and 1 ml of substrate was added to reaction mixture. After incubation at 25 °C for 1 h without shaking, 4 ml of 0.5 M NaOH and 0.5 M $CaCl_2$ were added for colour development. The reaction mixture was centrifuged at 2500 rpm for 10 min and supernatant was used for spectroscopic determination of *p*NP released at 420 nm. Soil urease was determined by the method of Fawcett and Scott (1960). 0.5 g of soil sample was mixed with 2 ml of 0.1 M Phosphate buffer (pH 7.0) and 0.5 ml of aqueous solution of urea (6.4% w/v) was added to it. Reaction mixture was incubated at 25 °C for 90 min. After incubation, final volume was made to 10 ml using distilled water and ammonia released in the solution was measured. Dehydrogenase enzyme was assayed using triphenyl tetrazolium chloride (TTC) by the method of Casida et al. (1964). Briefly, 2 ml 1% solution of TTC in 0.1 M Tris buffer was mixed with 2 g of fresh soil and incubated in dark at 25 °C for 24 h. Released triphenyl formazan (TPF) in the reaction mixture was extracted using 1:1 ratio of N, N-dimethyl formamide and ethanol for 10 min. Mixture was centrifuged at

Please cite this article as: Jain, R., Pandey, A., (2016), http://dx.doi.org/10.1016/j.rhisph.2016.07.007