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EFFECT OF ELEVATED TEMPERATURE ON SOME FUNCTIONAL BACTERIA IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) RHIZOSPHERE AT DIFFERENT PHENOLOGICAL STAGES

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ABSTRACT
The present study was conducted to study the impact of elevated temperature in the present and future climate change scenarios on some functional bacterial populations in Groundnut (B-95) rhizosphere. Rhizosphere soil samples were collected and numbers of bacteria involved in nitrogen-fixing, phosphate-solubilizing and potassium-dissolving were measured with cultivation-dependent approaches. + 2-3 °C elevated temperature conditions from germination to maturity affected the functional bacterial populations in rhizosphere soil positively with 7.02%, 6.61% and 3.0% increase in NFB, PSB and PDB respectively at complete flowering compared to the ambient which could be positively correlated with various growth parameters ($p \leq 0.05$). The current finding suggested that the elevated temperature throughout the crop growth was found to be having positive effect on the numbers of functional bacteria in groundnut rhizosphere.

INTRODUCTION

Stresses both abiotic and biotic, are major constraints to agricultural production. Under stressed environment, plant growth is affected by a number of factors such as physiological disorders, susceptibility to diseases, hormonal and nutritional imbalance, ion toxicity, etc. (Nadeem *et al.*, 2014). Climate is the primary determining factor of agricultural productivity. Plant ecosystems, and hence crop yields, are determined by many environmental factors which may work either synergistically or antagonistically with other components in deciding crop yield (Waggoner, 1983). According to IPCC AR5 (2013) the globally averaged combined land and ocean surface temperature data show a warming of 0.85ºC over the period 1880 to 2012 and for the end of the 21st century it is *likely* to exceed 1.5ºC relative to 1850 to 1900.

Groundnut (*Arachis hypogaea* L.) is a leguminous crop and is the 4th most important oilseed crop and 13th most important food crop of the world. Besides this, groundnut is a thermo-sensitive plant which requires a long and warm growing season and short or prolonged periods of high temperature during reproductive development of groundnut are known to cause significant yield losses (Ketring, 1984; Ong, 1986; Wheeler *et al.*, 1997).

Groundnut rhizosphere is known to host a variety of functional bacteria. The rhizosphere is the region of soil which is distinctly influenced by plant roots and plant-produced materials (Dessaux *et al.*, 2009). Very important and significant interactions have been reported among soil, plant and microorganisms present in the rhizosphere (Antoun and Prevost, 2005) which may either be beneficial, harmful and/or neutral and can significantly influence plant growth and development (Adesemoye and Kloepper, 2009; Ahmad *et al.*, 2011; Lau and Lennon, 2011). Zaidi *et al.* (2009) hold the unparalleled physico-chemical and biological characteristics of the soils associated with the roots responsible for the increased population and activity together with enhanced homogeneity of microorganisms in the rhizosphere.

The microbial population present in this environment is relatively different from that of its surroundings due to the presence of root exudates that serve as a source of nutrition for microbial growth (Burdman *et al.*, 2000). These compounds secreted by plant roots act as chemical attractants for diverse and actively metabolizing soil microbial communities. The exudation of a wide range of chemical compounds modifies the physico-chemical properties of the soil and thus, regulates the structure of soil microbial community in the immediate vicinity of root surface (Dakora and Phillips, 2002). Plant root exudates constituting of several organic materials are transuded throughout plant-microorganism interaction, which are taken up by microorganisms associated with the roots as nutrients for their own survival. Both quantity as well as quality of root exudates are dependent on and get modified by the supply and pattern of nutrients available to plants, which in turn affect the behaviour of microorganisms in the rhizosphere (Gryndler, 2000).

Functional microbial groups participate in nitrogen,

phosphorus, carbon, sulphur and other chemical cycles, and occur in all types of environments. Besides controlling the biogeochemical cycling of nutrients, these microorganisms also help plants to grow more proficiently not only under conventional soil environment but also stressed surroundings (Wani *et al.,* 2008 and Khan *et al.,* 2009). Soil productivity and nutrient cycling, in general, get influenced by microbial communities inhabiting the soil and in a similar manner plant growth get influenced by the relationships between the biogeochemical cycling of nutrients and functional groups of microorganism (Anderson, 2003). And in turn the physiological status of the plant, the presence of microbes and the bearing of products from rhizobacteria influence exudate composition.

We hypothesized that in the present climate change scenario, elevated temperature conditions may lead to increase, decrease or modification in the exudates released by plant roots which may modify the ecosystem functioning including the population and role of these functional bacteria.

MATERIALS AND METHODS

Field design and sampling

The field experiments were conducted at Research farm of Indian Agricultural Research Institute (IARI), New Delhi using soil samples from crop rhizosphere grown in Small Tunnels. Groundnut crop, *Arachis hypogaea* L. (B-95) was sown in Small Tunnels with four treatments: ambient temperature condition from germination to maturity (T1), elevated temperature condition (+2-3ºC) from germination to flowering (T2), elevated temperature condition (+2-3ºC) from flowering to maturity (T3) and elevated temperature condition (+2-3ºC) from germination to maturity (T4).

Quantification of functional bacteria in rhizosphere soil

Quantification of bacteria was carried out using the method described by Hu *et al.*, 2008. Briefly, total Nitrogen-Fixing Bacteria (NFB), Phosphate-Solubilizing Bacteria (PSB) and Potassium-Dissolving Bacteria (PDB) were enumerated and isolated using 10-fold dilution plate technique. Soil suspensions were obtained by shaking 1g soil sample from roots surface with tightly adhering soil in test tubes containing 9 ml Phosphate Buffer Saline (PBS) (Kaushal and Kaushal, 2013). The resulting suspensions were serially diluted 10-folds and the colony-forming units (c.f.u) of functional bacteria in each sample were determined by spreading 100 μL aliquots of each dilution onto the surfaces of appropriate culture media. The media used to assay for different bacteria types were prepared as described by Hu *et al.*, 2008. The plates were incubated at 28ºC for 7 days for nitrogen-fixing bacteria, 3 days for phosphate-solubilizing bacteria and 2 days for potassium-dissolving bacteria.

Statistical analysis

Statistical differences between numbers of bacteria ($\log_{10}$ c.f.u g^{-1} dry soil) at each growth stage were determined by independent-sample *t* tests at the 5% significance level. The treatment (effect of temperature), crop growth stage (days after sowing) and treatment * crop growth stage interaction effects on the numbers ($\log_{10}$ c.f.u g^{-1} dry soil) of functional bacteria were tested and analysed by two-way analysis of variance (ANOVA) using a generalized linear model (GLM) procedure. All statistical analyses were performed with SAS.

RESULTS

In the present study, we evaluated the functional bacterial population in groundnut (B-95) rhizosphere. The numbers of nitrogen-fixing, phosphate-solubilizing and potassium-dissolving bacterial population were significantly different ($p < 0.001$) between the different temperature treatments (viz. T1-ambient temperature condition, T2-elevated temperature condition from germination to flowering, T3-elevated temperature condition from flowering to maturity and T4-elevated temperature condition from germination to maturity) as well as during all the various crop growth stages (Table 1).

Nitrogen-fixing bacteria (NFB)

The present investigation revealed that the population of nitrogen-fixing bacteria in T1 increased till 75 DAS (complete flowering) and then it started declining (Fig. 1 (a).). Similar trend was observed in other temperature treatments; however, the population in T4 and T2 showed 7.0% and 5.9% increase respectively during the same period. Number of colony forming units of nitrogen-fixing bacterial population differ between T1 and T3 till 75 DAS (complete flowering), nevertheless at 90 DAS (pod development stage), the population was significantly higher (6.9%) in T3 as compared to T1. Although the number of nodules also showed a significant increase in T4 as compared to the other three treatments but the size of the nodules was lesser than at T1 (Fig. 2 (f).). In treatment T2 at 90 DAS (pod development stage) and 105 DAS (at maturity) there was a significant decline in the number of nodules as compared to other treatment conditions.

Phosphate-solubilizing bacteria

There were significantly fewer phosphate-solubilizing bacteria in T1 than the other three treatments at complete flowering, pod development stage and at maturity. The number of phosphate-solubilizing bacteria was significantly higher in T4 than the other three treatments at all stages of crop growth. While the numbers of phosphate-solubilizing bacteria showed

Table 1: Generalized linear model results of the overall effects of temperature treatment and growth stages on the number of nitrogen-fixing bacteria, phosphate-solubilizing bacteria and potassium-dissolving bacteria in groundnut rhizosphere

Source of variation (by bacterial type)	df	F	P
Nitrogen-fixing bacteria			
Temperature	4	441.84	0.001
DAS	6	393.28	0.001
Temperature × DAS	24	20.37	0.001
Phosphate-solubilizing bacteria			
Temperature	4	325.21	0.001
DAS	6	218.96	0.001
Temperature × DAS	24	9.86	0.001
Potassium-dissolving bacteria			
Temperature	4	195.33	0.001
DAS	6	290.13	0.001
Temperature × DAS	24	20.69	0.001

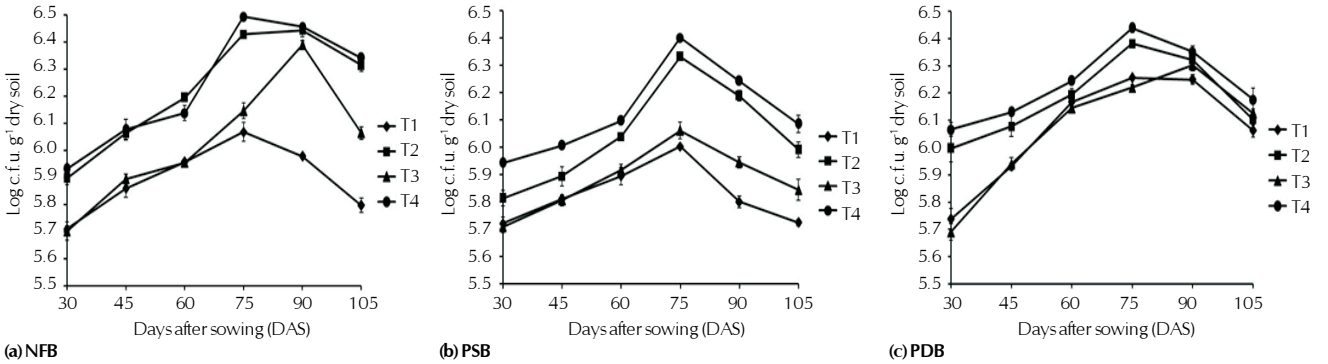

Figure 1: Dynamics of population of (a) Nitrogen-Fixing Bacteria (NFB), (b) Phosphate-Solubilizing Bacteria (PSB) and (c) Potassium-Dissolving bacteria (PDB) in groundnut (B-95) rhizosphere grown under ambient T1 (Germination to Maturity: ambient temperature) and above ambient temperature such as T2 (Germination to Flowering + 2-3°C), T3 (Flowering to Maturity + 2-3°C) and T4 (Germination to Maturity + 2-3°C) treatments. The data are the means of 4 replicates and error bars represent standard error

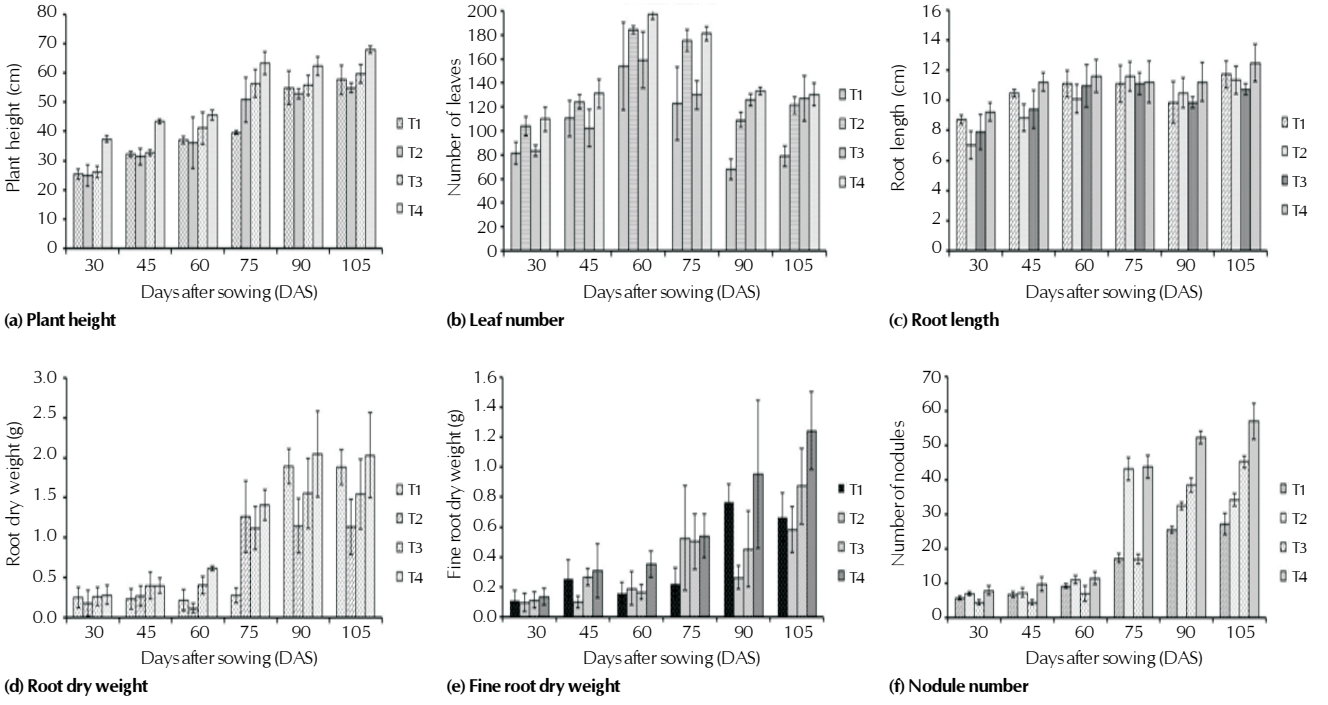

Figure 2: Effect of ambient T1 (Germination to Maturity: ambient temperature) and above ambient temperature such as T2 (Germination to Flowering + 2-3ºC), T3 (Flowering to Maturity + 2-3ºC) and T4 (Germination to Maturity + 2-3ºC) treatments (during different crop growth stages) on (a) Plant height , (b) Leaf Number, (c) Root length, (d) Root dry weight, (e) Fine root dry weight and (f) Nodule number of groundnut (B-95) grown in small tunnels. The data are the means of 4 replicates and error bars represent standard error

similar trends in all treatments over time they differed significantly at all crop growth stages. No consistent significant differences in the numbers of phosphate-solubilizing bacteria between T1 and T3 were observed at vegetative stage, initial flowering and 50% flowering, however, significant differences could be seen in the numbers of phosphate-solubilizing bacteria at complete flowering (75 DAS), pod development stage (90 DAS) and at maturity (105 DAS) between the two. In T4, there were significantly more phosphate-solubilizing bacteria than any other three treatments at all stages of crop growth and was 6.6%, 7.6% and 6.3% higher than T1 at complete flowering, pod development stage and at maturity respectively. In T3, there were no significant differences in the numbers of phosphate-solubilizing bacteria at vegetative stage, initial flowering and 50% flowering when compared to T1, however, there were significantly more phosphate-solubilizing bacteria in T3 than T1 at complete flowering, pod development stage and at maturity. Significant differences were observed in the numbers of phosphate-solubilizing bacteria in T2 and T4 at all growth stages when there were significantly more

phosphate-solubilizing bacteria in T4 than T2 (Fig. 1 (b).).

Potassium-dissolving bacteria (PDB)

Significantly less potassium-dissolving bacteria were observed in T1 and T3 than the other two treatments at vegetative stage (30 DAS) and initial flowering (45 DAS). The number of potassium-dissolving bacteria was significantly higher in T4 than the other three treatments at all stages of crop growth except for pod development stage (90 DAS) and at maturity (105 DAS), where although it was higher but non-significant. While the numbers of potassium-dissolving bacteria showed similar trends in all treatments over time the potassium-dissolving bacteria increased sharply from vegetative stage (30 DAS) to 50% flowering (60 DAS) in both T1 and T3 with 1.08 times increase. There was 5.7 % and 6.6% increase in T4 as compared to T1 and T3 respectively at vegetative stage (30 DAS). No consistent significant differences in the numbers of potassium-dissolving bacteria among all the treatments were observed at all crop growth stages, however in T4, there were significantly more potassium-dissolving bacteria than all the other three treatments at all stages of crop growth except for pod development stage (90 DAS) and at maturity (105 DAS). The potassium-dissolving bacterial population showed similar trend in T2 and T4 with significant differences ($p < 0.001$) except at initial flowering (45 DAS) and pod development stage (90 DAS). In T3, there were no significant differences in the numbers of potassium-dissolving bacteria at vegetative stage, initial flowering and 50% flowering when compared to T1, although, there were significantly more potassium-dissolving bacteria in T3 than T1 both at pod development stage and at maturity. Significant differences were observed in the numbers of potassium-dissolving bacteria in T2 and T4 at all growth stages except at pod development stage (Fig. 1 (c).).

Effect of temperature treatments on phenological and morphological parameters

The increase in the numbers of functional bacteria in treatment T4 be it NFB, PSB or PDB all was shown to correlate positively with the various phenological and morphological parameters. There was a significant variation ($p \leq 0.05$) in plant height, number of leaves, root length, root dry weight, fine root dry weight and number of nodules of groundnut in T4 when compared with T1, T2 and T3. Plants in elevated T4 recorded the highest readings in all the above phenological parameters at all sampling periods which support and explain the increased number of bacterial population. All the treatments showed similar trends over time. Elevated temperature condition throughout the growing season increased plant height with 1.6 times increase from the ambient at complete flowering (75 DAS) (Fig. 2 (a).). The root length of groundnut varied in T2, T3 and T4 when compared to T1 with no significant differences. Similar trends over time were observed in all the treatments. The maximum root length (12.5 cm) was recorded in T4 at maturity which was significantly 6.4% higher than the ambient (Fig. 2 (c).). The root dry weight of groundnut also varied in T2, T3 and T4 when compared to T1 with no significant differences. The maximum root dry weight (2.048 g) was recorded in T4 at pod development stage (Fig. 2 (d).) whereas the maximum fine root dry weight (1.24 g) was also recorded in T4 at maturity (Fig. 2 (e).). In addition, T4 showed the highest number of nodules at all sampling stages with maximum (57) at maturity (105 DAS). But a significant decline in the number of nodules could be seen in T2 from 75 DAS (complete flowering) to 90 DAS (pod development stage) (Fig 2 (f).).

DISCUSSION

Biological interactions between plant, soil and rhizosphere functional bacteria are believed to cause a cumulative effect on all rhizosphere components, and these interactions are also affected by environmental factors such as soil type, nutrition, moisture and temperature (Abdel Latef and Chaoxing, 2010; Nadeem *et al.*, 2014). It is evident from the literature that environmental stresses including both biotic and abiotic affect the plant growth and development by causing adverse effects on morphological, physiological and biochemical processes (Nadeem *et al.*, 2014). Microorganisms are sensitive to temperature and the present global warming scenario of 0.85ºC is most likely to influence microbial community structure and function and may be site dependent and respond differently to warming conditions (IPCC, 2013; Cregger *et al.*, 2014). Various experiments conducted indeed demonstrate the effect and regulation of temperature on soil microbial communities where it has been shown that their structure and function is directly regulated by temperature and indirectly by temperature effects on the aboveground plant community (Frey *et al.*, 2008; Schindlbacher *et al.*, 2011; Zogg *et al.*, 1997). Hence global warming may play a significant role in rapidly and dramatically altering the structure and function of soil communities. Therefore we studied the quantification of functional bacteria with different temperature treatments at different stages of crop growth and it's response to the phenology of groundnut.

The present study showed a consistent significant differences between ambient temperature condition (T1) and elevated temperature condition from germination to maturity (T4) in the numbers of culturable nitrogen-fixing bacteria, phosphate-solubilizing bacteria and potassium-dissolving bacteria during the six sampling stages. The numbers of bacterial populations were significantly higher in T4 (+2-3ºC elevated temperature condition from germination to maturity) in most of the instances except for a few where it was higher but non-significant.

Soil is a dynamic body and the soil microbial community structure is an early and sensitive indicator of biotic and abiotic effects on soil and plant ecology (Visser and Parkinson, 1992). It is a storehouse of various plant nutrients of which nitrogen is among the most common nutrients which is required in large quantities for desirable crop growth and development and although atmosphere contains 78% of N, yet it remains unavailable to growing plants. This is where microorganisms come into play. Nitrogen-fixing microorganisms, whether symbiotic, non-symbiotic or free-living, with the help nitrogenase which is a complex enzyme system, biologically fix nitrogen and convert unusable forms into plant utilizable forms (Kim and Rees, 1994) and help the plants in meeting their nitrogen requirements. We found more numbers of functional bacteria in T4 as compared to T1. Studies show that functional bacteria help in stress alleviation by nitrogen fixation (Berg, 2009; Hayat *et al.*, 2010).

Apart from nitrogen, phosphorus is also one of the most

essential nutrients for plant growth. Phosphorus is involved in numerous important plant metabolic processes which include energy transfer, signal transduction, macromolecular biosynthesis, respiration and photosynthesis etc. (Shenoy and Kalagudi, 2005). Regrettably, phosphorus is one of the least available and the least mobile mineral nutrients for plants in the soil (Takahashi and Anwar, 2007), hence decreasing the efficiency with which phosphorus can be taken up by the plants. Phosphate-solubilizing microorganisms play central roles in nutrient cycling of phosphorus in both natural and agricultural ecosystems. Delvasto *et al.* (2006) have suggested various ways by which phosphate-solubilizing bacteria are able to transform insoluble phosphorus to soluble forms. However, all phosphate-solubilizing microbes studied to date are mesophilic in nature and do not work at the high temperatures (over 50ºC) although phosphate-solubilizing bacteria and proteolytic microbes have been shown to increase rapidly up to 32ºC (Yang and Chen, 2003). In a different study it has also been shown that identification and characterization of soil PSBs for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field (Guleria *et al.,* 2014). We recorded increased numbers of phosphate solubilizing bacteria in T4 than T1.

Potassium is another essential macronutrient which plays a crucial role in the growth and development of plants for it is a key element in many physiological and biochemical processes. It is known to activate enzymes, enhance photosynthesis, reduce respiration, help in nitrogen uptake, maintain cell turgor, help in transport of sugars and starches and is necessary for protein synthesis. It improves crop quality by strengthening straw, helping in grain filling and kernel weight and increasing disease resistance to help the plant to withstand stress. Potassium-dissolving bacteria have been found to dissolve silicate minerals but their activity is restricted to a certain temperature supporting which Sheng *et al.* (2002) have reported 35.2 mg/L potassium release after 7 days of incubation at 28ºC from strains of potassium-dissolving bacteria in pH ranging from 6.5-8.0.

The reason behind our results can be attributed to the fact that with an increase in temperature, as has been predicted under future climate conditions, root exudation of organic C also increases as shown by Uselman *et al.* (2000). They found that elevated atmospheric CO_2 did not affect root exudation of organic C by *Robinia pseudoacacia*. However, warmer climates, as predicted for the next century, may accelerate root exudation of organic C and under the present climate change scenario the role of root exudation may be slightly altered (Uselman *et al.*, 2000). Their results showed that as temperature increases, as has been predicted under future climate conditions, root exudation of organic C also increases by a factor of 1.7. This implies that exudation of organic C may be an active metabolic process, stimulated by the increase in temperature and hence about 7.02% increase in the number of nitrogen-fixing bacteria, 6.61% in phosphate-solubilizing bacteria and 3% in potassium-dissolving bacteria in T4 than in T1 can be explained due to the increase in quantity of and change in the composition of root exudates.

In a similar study, Zogg *et al.* (1997) found that microbial respiration increased dramatically with soil warming due to an apparent increase in the pool size of C metabolized by soil microbes at higher temperatures. One plausible mechanism for this response can be due to a temperature-induced shift in microbial community composition, wherein dominant populations at higher temperatures have the ability to metabolize substrates that are not utilized by members of the microbial community at lower temperatures. They examined the effects of temperature on the composition and function of microbial communities, in conjunction with an examination of the kinetics of microbial respiration and showed that the apparent increase in substrate pool size at higher temperatures is a shift in microbial community composition associated with soil warming.

It is widely recognized that certain groups of soil microorganisms are well adapted to particular temperature regimes. Furthermore, soil microbes can vary considerably in their affinity for different substrates. Therefore, if temperature elicits changes in community composition, the dominant populations may be favoured at higher temperatures which may metabolize the increased amount of substrates and colonize the rhizosphere in increased numbers (Zogg *et al.*, 1997). However, we cannot deduce it from here if the same bacterial population prevailed at all crop growth stages or there has been a shift in the microbial community due to temperature treatments.

In conclusion, the microbial populations dwelling in the soil environment are not regulated by a single factor as temperature. Besides, the global climate change does not solely involve temperature but also carbon dioxide, with other factors such as pH, soil moisture etc. also playing an important role in determining microbial populations in soil. Therefore, it appears that although we could find increase in the populations of functional bacterial with +2-3ºC increase in temperature, this increase might or might not be in unification with other factors. Hence, additional research on the effects of temperature on patterns of microbial populations and communities is clearly necessary in order to confirm our conclusions and to better understand the dynamics of soil microbial community under future climate warming scenarios.

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