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Effect of elevated temperature on soil microbial activity and nitrogen transformations in wheat crop (*Triticum aestivum*)

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ABSTRACT

Wheat (*Triticum aestivum* L.), a major staple crop in India is susceptible to climatic variability including elevated temperature and altered precipitation patterns. The increase in atmospheric temperature has a profound impact on wheat crop production as well as below ground nutrient transformations. Field experiment was conducted under elevated temperature conditions, to understand the possible effects of elevated temperature on soil microbial activity, biomass and soil nitrogen transformations in wheat crop. The experimental crop was grown in four separate tunnels having an average per degree increase in temperature in each tunnel up to 3°C ± 0.5°C above the atmospheric temperature. Various biological and physical parameters of soil, including N-cycling microbial population (ammonifiers and nitrifiers), microbial biomass carbon (MBC) and nitrogen (MBN), soil organic carbon (SOC), soil NH_4^+-N and NO_3^--N, potential nitrogen mineralization (PNM), denitrification, along with crop yield and grain nitrogen were determined at different phases of crop growth. Although, any significant variation in N cycling microbial populations (ammonifiers and nitrifiers) was not observed with respect to elevated temperature but rise in soil NO_3^--N, PNM, MBC by 27, 21, and 18%, respectively, was recorded in T4 treatment(+ 3°C elevation). Denitrification as indicated by nitrate reductase activity increased by two-fold under the warmer conditions. Our results suggest that the warmer climatic conditions favour net N mineralization rather than its immobilization in soil system.

Key words: Denitrification, Elevated temperature, Mineralization, N-cycling bacteria, Nitrification.

Anthropogenic activities across the globe have led to increase in greenhouse gases concentration, thereby leading to rising temperature, altered rainfall patterns and extreme weather events. As climate is a prime determinant of agricultural productivity, any variations in climate, influences the agriculture sector, which is backbone of the economy of developing countries such as India. Elevation in temperature and CO_2 associated with the changing climate have pernicious effects on agro-ecosystem, especially on agricultural productivity, crop growth and yield of major staple crops all over the world. The projected impact of climate change particularly in India is an increase in average temperature by 2-4°C and irregular rainfall in monsoon and winter months further causing floods and draughts in different regions.

Due to the fluctuations in growing season temperature over the years which seem to be affecting yearly wheat yield, with every 1°C rise in temperature. The country's annual wheat productivity could decline by 6 million tonnes (Aggarwal and Swaroopa Rani 2009). Wheat is very sensitive to higher temperatures, heat stress during the reproductive phase is more devastating as compared to vegetative phase because of the direct effect on grain number and dry weight.

To deal with these changes in climate, there is imperative need to make the cropping system adaptable to future climate, through modifications in the root/soil interactions, which may help plants to survive harsh or stressed conditions. For this, a better understanding of below ground processes carried out by microorganisms in response to climate change is needed. They play a key role in regulating organic matter decomposition and plant nutrient availability, and also provide adaptability and stress tolerance

Microbes are one of the driving force of major N transformation in soils, i.e. mineralization, nitrification, immobilization, and denitrification. Changing climate scenario may affect the microbial community dynamics involved in bio-geochemical cycling and hence may potentially alter microbially mediated nitrification and denitrification dynamics in the soil ecosystem. The real dynamics of the effects of climate change on microbial communities and their role in nutrient transformations can be studied in field experiments where other ecosystem

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also play a pivoted role. Our study thus focuses on how elevated temperature under predicted climate change scenario alters the microbial community dynamics and nitrogen transformations, which may have implications on the N mineralization or immobilization of the soil system.

MATERIALS AND METHODS

The experimental site, research farm of Indian Agricultural Research Institute, New Delhi falls in the Indo-Gangetic alluvial tract at 28^0 40'N and $77^0$12'E, at an altitude of 228 m above mean sea level. The climate of New Delhi is subtropical semiarid with average rainfall of 740 mm, about 80% of which occurs from July to September. The soil characteristics of the experimental site are sandy loam with 66.4% sand, 14.9% silt and 17.1 % clay and bulk density of 1.53 g cm^3, and pH 7.6 (1:2 soil: water).

Wheat (variety HD 2967) was grown from November 2012 to April 2013. The experimental crop was grown in tunnels of size 10 × 2.5m using solar radiations as light source. The temperature tunnels namely T1 (as control) having atmospheric ± 0.5°C, T2, T3, T4 having an average per degree increase of temperature respectively in every tunnel up to 3°C ± 0.5°C above the atmospheric temperature were maintained throughout the crop growth period. Ascending elevated temperature in tunnels was maintained during the entire course of crop duration through hot air blowers. These tunnels were equipped with sensors for temperature recording by data loggers and daily log of atmospheric as well as tunnel temperature was maintained. All cultural practices, viz sowing, thinning, weed management, irrigation and harvesting were carried out as per standard procedure. Along with the basal dose of diammonium phosphate (DAP, 150 kg/ha), urea was applied at the rate of 120 kg N/ha in three equal splits at sowing (basal), crown root initiation stage (21 DAS), and panicle initiation stages (45 DAS).

Soil samples were collected from 0-15 cm depth with a 2 cm diameter auger from five locations in the plot at four stages of crop development, i.e. vegetative, flowering, grain filling, and harvest (28, 61, 92, 128 DAS, respectively). Sample were homogenised and subjected to analysis for different soil physicochemical parameters using standard procedures. For microbiological analysis, rhizospheric soil was collected in sterilized plastic bags and stored at 4°C till further analysis. For plant N determination, leaves, stem, and spikelet (grains at harvest) were collected at flowering and harvest stage (92, 128 DAS).

The inorganic N, viz. $NH4^+$ and $NO3^-$ content were analyzed by 2 M KCl extraction method and UV spectroscopy method, respectively (Keeney and Nelson 1982, Celsceri *et al*. 1998). The available Nitrogen in soil was estimated by alkaline permanganate procedure as described by Subbiah and Asija (1956), and total Nitrogen content (inorganic + organic) in soil samples was assessed by Kjeldhal method.

Soil organic carbon (SOC) was estimated by Walkley and Black method (1934). Soil microbial biomass carbon (MBC) and nitrogen (MBN) were estimated using the chloroform fumigation-extraction method (Vance *et al*. 1987), with the efficiency factor as 0.45 K_{EC} for MBC (Vance *et al*. 1987) and 0.54 K_{EN} for MBN (Brookes *et al*. 1985).

Aerobic incubation method was employed for determining potential nitrogen mineralization of soil. A 10 g soil sample was mixed with 6 ml water and incubated at 30°C for 14 days under aerobic conditions. After incubation, samples were extracted with 2M KCl solution, and distilled in Kjeldhal system. The extractable NO_3-N and NH_4-N were estimated and calculated as outlined by Keeney and Bremner (1966).

For determination of nitrate reductase activity (NRA) fresh soil was incubated in polypropylene bottles with known amount of NO_3^--N for 24 hr at 28°C. After incubation sample was extracted with 2.5M KCl solution. A portion of extracted sample was mixed with diazotizing and coupling reagent, and analyzed at 540 nm. The enzyme activity was calculated using and using standard NO_2^- -N as reference as indicated by Roberge *et al*. (1978).

Population of microbes involved in nitrogen cycling were evaluated by the Most Probable Number (MPN) method on 96 well plate (Rowe *et al*. 1977). Ammonifiers were enumerated in N-free winogradsky's saline solution plus oligo-elements. Ammonium and nitrite calcium carbonate medium was used for culturing Ammonia-Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB), respectively (Alexander and Clark 1965). MPN value from appropriate table was expressed as $\log_{10}$ MPN/g dry soil after applying correction for the initial dilution and inoculants volume. Data were statistically analyzed following standard statistical methods (Gomez and Gomez 1984) using MS Excel and SPSS 20. Values reported are the mean of five replicate. Unless otherwise stated, the level of significance referred to in the results is $P < 0.05$.

RESULTS AND DISCUSSION

To investigate the impacts of elevated temperature on below ground processes temperature tunnel experiment was set up. During the season mean monthly minimum and maximum atmospheric temperature varied from 11.1° to 36.2°C, and in control tunnel it ranged from 11.6° to 38.7°C.

Microbial dynamics involved in N-cycling

The growth curve of different microbes, i.e. ammonifiers, AOB, NOB, followed similar pattern from T1 to T4 treatment. The maximum mean density of microbes was observed at the flowering stage irrespective of the treatment, followed by a decline at harvest stage. The marked increase in soil microbial population during the transition of the crop from vegetative phase to reproductive phase is presumably due to the presence of available nutrient sources and magnification factors in root exudates and sloughed root cells.

The population of ammonifiers at T1 treatment ranged from 6.076 MPN/g soil ($\log_{10}$ values) at vegetative stage, with its maximum value of 7.7 MPN/g soil (gaining 26.7%) during flowering stage, declining to 4.816 MPN/g soil at

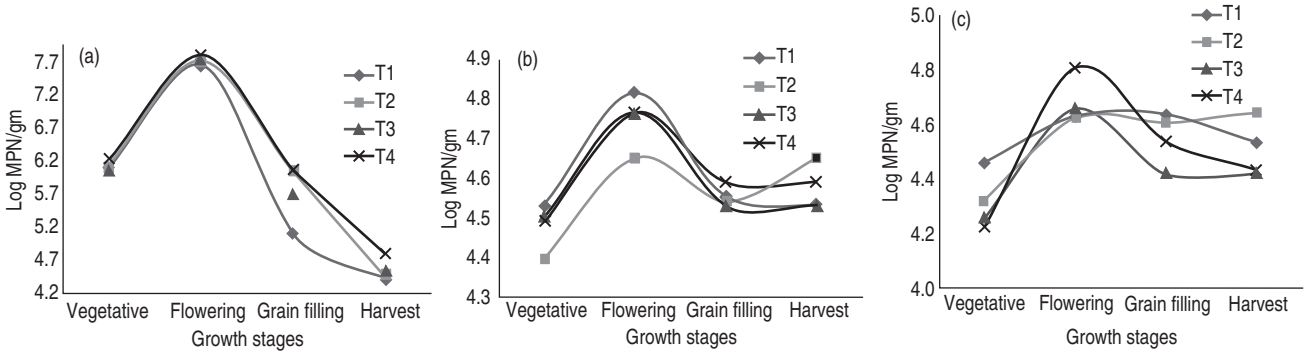

Fig 1 Growth pattern of N-cycling bacteria as effected by elevated temperatures. (a) Ammonifying bacteria, (b) Ammonium oxidizing bacteria, (c) Nitrite oxidizing bacteria.

harvest. The population density for ammonifiers in T2 treatment was 0.5 to 5% higher at all growth stages as compared to T1. Similarly, rise in population densities, markedly in the grain filling stages of T3 and T4 treatment (11 and 19%, respectively) as to T1 was recorded (Fig 1).

The microbial population of AOB and NOB were reported as 2-3 logarithmic units (10^4) lower than ammonifiers. In T1 treatment, at flowering stage, AOB exhibited a maximum density of 4.816 MPN/g soil (an increase of 6.2% from its vegetative stage), which declined to 4.531 MPN/g soil at harvest. Correspondingly, the population size of AOB in T2, T3, and T4 treatment attained lower values than T1 at vegetative and flowering stages.

NOB reached a maximum population of 4.637 MPN/g soil at flowering and grain filling stage under T1 treatment. Analogous to AOB, the variations recorded in NOB population are inconsequential. Only under flowering stage of T4 treatment, NOB registered a 3.8% increase in bacterial population compared to T1 treatment. With per degree increase in temperature, though the ammonifier's population demonstrated a positive response, the nitrifying bacterial population including AOB and NOB exhibited an insignificant variation. Our results corroborated the findings of Rakshit *et al*. (2012), who also reported non-consequential variation in microbial population under elevated temperature treatment in wheat crop grown in pot.

The decrease in nitrifiers population could be attributed to the possible limitation of population, by rate of production of ammonia. Urea used as a fertilizer can also cause some decline in AOB population (Martikainen 1985). With the traditional MPN method, we could gather only the variation in the size of different population, however, how the climate change is impacting the different groups of bacteria within that population can be checked through the molecular techniques like PCR and microarray etc.

Impact on microbial biomass carbon and nitrogen

The microbial biomass carbon (MBC) depicted a positive effect with rise in temperature. In T1 treatment, the MBC value showed an upsurge from 371 mg/kg (at vegetative stage) to 399 mg/kg at flowering and to 405 mg/kg at grain filling stage of crop development. The MBC was highest at grain filing stage for all temperature treatment (Table 1). Significant increase of 13% ($P < 0.05$) and 18% rise ($P < 0.05$) as compared to T1 was observed in T3 and T4 treatment, respectively, during flowering phase. Treatment T4 registered significant variations to T1 (Table 1) at all growth stages of crop. The results indicate a positive response of microbial biomass to elevated temperature with abundant water and N availability. However, no significant variation was observed in MBN and SOC under elevated temperature conditions (data not shown).

Microbial biomass levels in soil are influenced by the soil organic carbon and in some cases have been shown to be inversely proportional to soil carbon/nitrogen ratio. The recorded increase in MBC without any apparent rise in soil carbon was possibly due to the increased oxidative enzyme activity that allows soil microbes to access 'recalcitrant' soil organic matter more than 'labile' soil organic matter under warmer climate conditions (Bauer *et al*. 2008). The increase in MBC initially with elevated temperature might be transitory, and with time warming may have negative effect on MBC due to the altered microbial growth efficiency. Wardle and Parkinson (1990) also reported observed positive relation between microbial biomass and temperature during the growing season, when effects of moisture variation are controlled.

Though, no significant difference in MBN values was observed, however, it is reported to decline under fertilization treatment and also due to drainage in fertilized lands (Bardgett *et al*. 1999).

Soil available N

Nitrogen transformations are primarily carried out through biological activities, which are further determined by climatic conditions and hence susceptible to temperature fluctuations. With an average per degree increase in temperature, no significant variations was observed in the total nitrogen. On the other hand, available nitrogen recorded a significant rise of 9.6%, and 16.9% during vegetative phase of crop growth in T3 and T4, respectively (Table 1).

Favourable impact of warming was observed on ammonium (NH_4^+-N) and nitrate (NO_3^--N) nitrogen as well. As compared to control, NH_4^+-N gained by 6% (P

<0.01) and 6.4% (P<0.01) at flowering phase in T3 and T4, respectively. Subsequently, the extractable NO_3^--N under T3 registered a 14%, 13% (P<0.01) and 4% (P<0.01) increase for vegetative, flowering and grain filling phase respectively. In final elevated temperature treatment, i.e. T4, a rise of 21%, 24% (P<0.01), and 17.4% (P<0.01) in NO_3^--N during vegetative, flowering and grain filling phases of crop development, respectively was observed (Table 1).

Warmer conditions positively influenced the soil N-availability (NH_4^+-N and NO_3^-N); although no paramount variation in AOB and NOB population was observed. The considerable increase in available nitrogen due to higher mineralization clearly points towards an increased microbial activity rather the population size. Apart from the microbial processes, the observed increase in nitrification could either be a consequence of favoured oxidation capacity of soil or proportionally higher gross N mineralization rather than immobilization. Griffin and Honeycutt (2000) reported that nitrification increases linearly with increasing temperature till 40°C and deceases at or above 45°C. Wang *et al*. (2006), also reported higher soil nitrogen availability under warmer conditions due to higher rates of mineralization. When elevated temperature alone was given as stress, there was marked increase in soil nitrogen availability but the soil carbon remained unaltered, indicating that soil system favoring low C/N ratio. Under multi-factorial experiment set up, including elevated CO_2 and altered precipitation, along with the elevated temperature, the soil system may behave differently.

Soil potential nitrogen mineralization (PNM)

PNM of soil was positively influenced by rising temperature, as higher values were observed under elevated temperature compared to control. Over the flowering phase, rate of nitrogen mineralization increased by 12% in T2, 17% in T3 (P<0.05), and 21% in T4 (P<0.01). Similarly, around 30% (P<0.05) rise in nitrogen mineralization was observed in T3 at grain filling phase.

Furthermore, the observed rise in Nitrate-N could also be the consequence of elevated PNM rate. There can also be a few indirect but co-relating explanations to the excess nitrates. As the mean temperature for crop was higher, the crop growth and its development were advanced during vegetative stages, thereby shortening its growing cycle by 9 days. The vegetative stage was shortened by 3, 7 and 9 days in T2, T3, T4, respectively. This fastened development and shortened growing period may have restricted N uptake by the wheat crop much earlier, leaving more N in soil, compared of the crop grown under ambient conditions, thus making more of N available in soil.

The increase in N mineralization under warmer conditions could be due to the increased rate of substrate decomposition considering the high microbial activity (Schindlbacher *et al*. 2011) and rising MBC. The higher availability of ammonium ions in the system also accounts for the subsequent increase in potential nitrification and further raising the PNM.

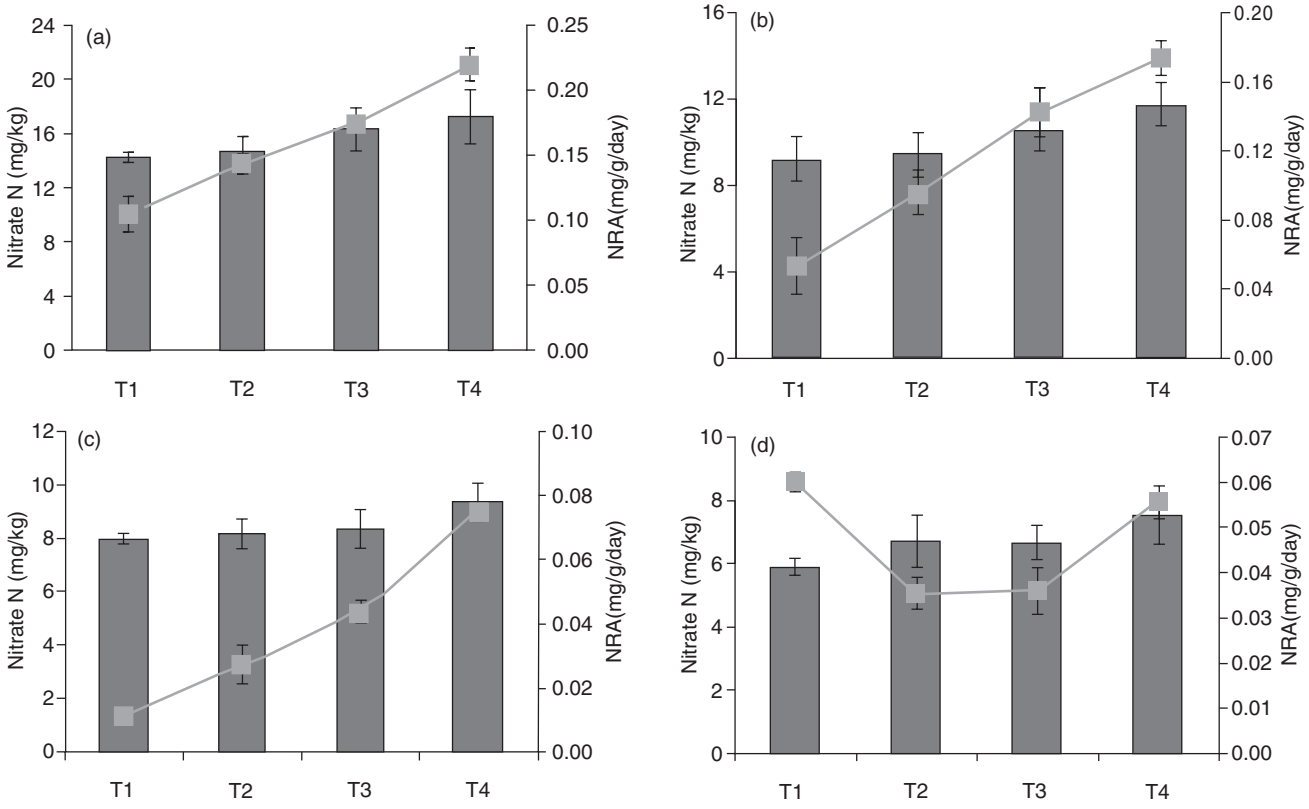

Fig 2 Nitrate –N and Nitrate reductase activity in relation to elevated temperature at (a) vegetative, (b) flowering, (c) grain filling and (d) harvest stage of crop development

Table 1 Effect of elevated temperature on available N, NH_4^+-N, NO_3^-N, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), potential nitrogen mineralization (PNM), nitrate reductase activity (NRA). Values with the same letter within the column (at each stage) are not significantly different at $P < 0.05$.

Growth stage and treatment	MBC (mg/kg)	Available N (mg/kg)	NH_4^+-N (mg/kg)	NO_3^-N (mg/kg)	PNM (mg/kg/day)	NRA (mg/kg/day)
Vegetative						
T1	372 [a]	249.7 [a]	6.31 [a]	14.26 [a]	1.653 [a]	0.1048 [a]
T2	378 [ab]	261.6 [ab]	6.45 [ab]	14.67 [a]	1.727 [ad]	0.1437 [a]
T3	403 [ab]	273.6 [b]	6.48 [ab]	16.32 [a]	1.850 [ab]	0.1745 [b]
T4	425 [bc]	291.8 [c]	6.62 [b]	17.22 [a]	2.023 [b]	0.2195 [c]
Flowering						
T1	399 [a]	137.4 [a]	6.14 [a]	9.24 [a]	1.840 [a]	0.0533 [a]
T2	395 [a]	137.3 [a]	6.34 [ab]	9.43 [ad]	2.061 [ab]	0.0960 [ab]
T3	452 [b]	136.8 [a]	6.51 [b]	10.61 [b]	2.167 [b]	0.1423 [b]
T4	470 [b]	144.0 [a]	6.53 [b]	11.75 [c]	2.229 [b]	0.1740 [c]
Grain Filling						
T1	405 [a]	135.0 [ab]	5.70 [a]	8.00 [a]	1.722 [a]	0.0113 [a]
T2	408 [ab]	135.7 [ab]	5.80 [a]	8.20 [ab]	1.823 [a]	0.0272 [b]
T3	453 [a]	130.5 [a]	5.71 [a]	8.37 [b]	2.254 [b]	0.0439 [c]
T4	479 [bc]	142.5 [b]	5.90 [a]	9.39 [c]	2.162 [ab]	0.0751 [d]
Harvest						
T1	358 [ab]	137.0 [a]	5.80 [a]	5.90 [a]	1.591 [a]	0.0601 [a]
T2	324 [ab]	132.4 [a]	5.78 [a]	6.72 [a]	1.627 [ab]	0.0353 [b]
T3	404 [a]	136.1 [a]	5.88 [a]	6.69 [a]	1.670 [ab]	0.0360 [b]
T4	430 [b]	139.6 [a]	6.15 [a]	7.54 [a]	1.813 [c]	0.0557 [ac]

Nitrate reductase activity

Significant effect of elevated temperature on the nitrate reductase enzyme activity (indicating denitrification) was observed. Enzyme activity rose successively from 0.104 mg/g/day in T1 to 0.219 mg/g/day in T4 treatment, amid vegetative phase. Furthermore, the nitrate reductase activity at flowering stage showed approximately three times upsurge from T1 to T4 (Table 1). Similar pattern was exhibited in later stages of crop development indicating an accelerated loss of nitrogen from soil.

As denitrification is a microbially mediated process therefore, higher rates of denitrification have been reported at higher temperatures. Our results showed high correlation value of nitrate availability and NRA (correlation value of 0.9195), which indicated that the higher rate of denitrification can be attributed to increased availability of nitrate ions (Fig 2).

As inorganic N (a major source of plant-available N), generated by the mineralization of organic N compounds acts as the substrate for the dentrifiers, their higher availability also may serve as the initiation point for the potential loss of N (Luce *et al*. 2011). The excess nitrate ions under elevated temperature can also cause increased N_2O emission from the agricultural soils due to denitrification. With increasing temperatures, N_2O gas fluxes are expected to rise and may add up to a positive feedback to global warming in the future.

Crop yield

In spite of the increased availability of nitrogen in soil, plant yield declined by 33% at T2, 37% at T3, and 40% at T4. Warming by only a few degrees has negative impact on plant nutrient use efficiency, carbon fixation, biomass accumulation, and yield. The negative impact of elevated temperature inclines to be larger on grains yield as compared to total biomass and grain nitrogen (Prasad *et al*. 2011). The possible explanation of the observed yield decline could be; shortened growth period of crop, increased denitrification, temperature sensitivity of crop particularly at grain filling stage.

This study was carried out to look into the effect of warmer conditions (as a consequence of climate change) on microbial population dynamic and their consequences on biochemical transformations. No significant effect of warming on N-cycling bacteria was observed, although microbial biomass responded positively to elevated temperatures. Warmer conditions stimulated soil N

availability by accelerating rates of mineralization. However, the N demand by the plant was not synchronized with N supply (due to shortening of growth period) which, lead to higher system N losses. Our results indicated that elevated temperature favours low C/N ratio in soil, thereby indicating more N mineralization as compared to immobilization. The high nitrogen availability (which ultimately causes higher N losses) under warmer conditions also suggest that the amount of fertilizers used during the crop growth and their application at different stages should be reviewed to prevent additional losses of N under elevated temperature. To minimize the effect of elevated temperature on winter wheat, varieties having longer vegetative period and normal reproductive phase can be tried on field. As all experiments were conducted to simulate the elevated temperature conditions under climate change scenario, certain negative affects observed in this study may get nullified in the combined effect of elevated temperature, CO_2 and altered precipitation. Further studies are therefore needed to analyze the combinatorial effects of various conditions that are expected to change as a result of climate change.

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REFERENCES

Aggarwal P K and Swaroopa Rani D N. 2009. Assessment of climate change impacts on wheat production in India. *(In) Global climate change and Indian agriculture*, pp 9. Case studies from the ICAR Network Project, Indian Council of Agricultural Research, New Delhi.

Alexander M and Clark F E. 1965. Nitrifying bacteria. (*In*) *Methods of soil analysis, Part 2*. pp 1 477–83. Black CA *et al*. (Ed). American Society of Agronomy, Madison, Wisconsin.

Bauer J, Kirschbaum M U F, Weihermüller L, Huisman J A, Herbst M and Vereecken H. 2008. Temperature response of wheat decomposition is more complex than the common approaches of most multi-pool models. *Soil Biology and Biochemistry* **40**(11)**:** 2 780–6.

Bardgett R D, Lovell R D, Hobbs P J and Jarvis S C. 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. *Soil Biology and Biochemistry* **31**(7)**:** 1021–30.

Brookes P C, Landman A, Pruden G and Jenkinson D S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* **17**(6)**:** 837–42.

Celsceri L S, Greenberg A E and Eaton A D. 1998. Standard Method for Examination of Water and Wastewater, 20th Edn. American Public Health Association, Baltimore, MD.

Gomez K A and Gomez A A. 1984. *Statistical Procedures for Agriculture Research*. A Wiley-Inter Science Publication. New York.

Griffin T S and Honeycutt C W. 2000. Using growing degree days to predict nitrogen availability from livestock manures. *Soil Science Society of America Journal* **64**(5)**:** 1 876–82.

Keeney D R and Bremner J M. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal* **58**(5)**:** 498–503.

Keeney D R and Nelson D. 1982. Nitrogen—inorganic forms. (*In*) *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, pp 643–98.

Luce M S, Whalen J K, Ziadi N and Zebarth B J. 2011. Nitrogen dynamics and indices to predict soil nitrogen supply in humid temperate soils. *Advances in Agronomy* **112**(55).

Martikainen P J. 1985. Numbers of autotrophic nitrifiers and nitrification in fertilized forest soil. *Soil Biology and Biochemistry* **17**(2)**:** 245–8.

Prasad P V V, Pisipati S R, Momčilović I and Ristic Z. 2011. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. *Journal of Agronomy and Crop Science* **197**(6)**:** 430–41.

Rakshit R, Patra A K, Pal D, Kumar M and Singh R. 2012. Effect of elevated CO2 and temperature on nitrogen dynamics and microbial activity during wheat (Triticum aestivum L.) growth on a subtropical inceptisol in India. *Journal of Agronomy and Crop Science* **198**(6)**:** 452–65.

Roberge M R. 1978. Methodology of soil enzyme measurement and extraction. *(In) Soil Enzymes*, pp 341–70. Burn R G, (Ed). Academic Press, New York.

Rowe R, Todd R and Waide J. 1977. Microtechnique for most-probable-number analysis. *Applied and Environmental Microbiology* **33**(3)**:** 675–80.

Schindlbacher A, Rodler A, Kuffner M, Kitzler B, Sessitsch A and Zechmeister-Boltenstern S. 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology and Biochemistry* **43**(7)**:** 1 417–25.

Subbiah B V and Asija G L. 1956. A rapid procedure for the estimation of available nitrogen in soils. *Current science* **25**(8)**:** 259–60.

Vance E D, Brookes P C and Jenkinson D S. 1987. An extraction method for measuring soil microbial biomass C. *Soil biology and Biochemistry* **19**(6)**:** 703–7.

Wang C, Wan S, Xing X, Zhang L and Han X. 2006. Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biology and Biochemistry* **38**(5)**:** 1 101–10.

Walkley A and Black I A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science* **37**(1)**:** 29–38.

Wardle D A and Parkinson D. 1990. Interactions between microclimatic variables and the soil microbial biomass. *Biology and Fertility of Soils* **9**(3)**:** 273–80.