

Abiotic stress affects resistance and resilience capacity of soil respiration under long-term manuring and fertilization in an Alfisol

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ABSTRACT

Present study was undertaken to evaluate the impact of long-term fertilizers application on substrate induced soil respiration (SIR), their resistance and resilience against heat stress (48°C for 24 h) of an acidic soil under sub-humid climate. To achieve this, soil samples (0-15 cm depth) were collected from five treatments viz. control, 100% NP (NP), 100%NPK (NPK), 100% NPK+FYM (NPKF) and 100% NPK + lime (NPKL) from long-term fertilizer experiment (LTFE), Birsa Agricultural University, Ranchi (since 1971) at pod formation stage of soybean crop (2018). Resistance and resilience capacity of soil SIR being analyzed at 1 and 30 days after heat stress (DAS). Results revealed that, SIR was significantly affected by long-term fertilization and heat stress. Balanced fertilization along with lime application (NPKL) had significantly higher SIR rate (10.78 mg CO₂ g/soil/h) followed NPKF, NPK, control and NP treatments. After 1 DAS, the SIR increased at 24 hour of substrate addition. However, during the 24-48 hours, the SIR value decreased significantly and maximum reduction was noticed under control treatments (47%). Cumulative SIR decreased significantly after heat stress, maximum reduction being in NPKL and NPKF treatments, whereas minimum under control. NPKF = NPKL treatments recorded highest resistance (0.86) followed by NP (0.82) > NPK (0.79) > control (0.61). Resilience index of NPKL (0.52) and NPKF (0.50) were highest and superior over other treatments. In nutshell, SIR emerged as a good indicator to assess resistance, resilience capacity of soil against heat stress.

Keywords: Soil respiration, heat stress, acidic soil, lime, long-term fertilization,

INTRODUCTION

Soil respiration is used to measure the activity of microbial groups in soil and is a critical component of soil ecosystem functioning as well as carbon storage. Soil respiration measurements can help to inform carbon sequestration efforts under climate change scenario, while also providing a means to monitor the health, and overall function of agricultural soils. The immediate increase in the carbon dioxide production by microorganisms to the addition of an easily degradable carbon source, i.e. the substrate induced respiration (SIR), is an indirect estimate of the microbial biomass and microbial activity. Soil respiration and SIR can be measured either in the field, where it is subject to natural conditions, or the laboratory, where conditions can be controlled with more accuracy (Allison *et al.* 2008; Kumar *et al.* 2014). The substrate induced soil respiration controlled by several factors viz. temperature, moisture, nutrient content and level of oxygen in the soil can produce extremely

disparate rates of respiration. In present scenario of climate change, soil resistance and resilience is a vital concept in retaining sustainability (Vishwanath *et al.* 2021; Kumar *et al.* 2013) and it is agreeable that resistant and/or resilient soil systems are desirable for sustainable crop production. Resistance and resilience, of microbial community to environmental stresses (e.g. high temperature, drought events etc.) directly/indirectly influences the maintenance of soil functions and consequently the soil quality sustainability (Kumar *et al.* 2014). Although, studies have shown the separate impacts of climate change on biogeochemical cycles, and on the composition of microbial communities. However, the effects of sudden increase in temperature during summer month especially in sub humid climate have been less investigated on substrate induced soil respiration, in relation with their intensity and duration. Change in soil fertility patterns and yield trends in different soil type have been recorded in recent years in many long-term fertilizer experiments (Behera and

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Singh, 2010; Vishwanath *et al.* 2020). Many studies reported that inorganic fertilizer application resulted in significant increases in SOC and its fractions due to the positive effects of the fertilizer on crop growth and in turn crop C return (Gong *et al.* 2009). Mandalet *al.* (2007) reported that microbial biomass can be controlled long-term by application of fertilizer and manure. However, only limited data on microbial activity and abundance have very important role in regulating biochemical processes, which are responsible for cycling of different nutrient, maintenance of soil health and resilience and resistance of the system against abiotic and biotic stress. The aims of this study were to assess the resistance and resilience of soil respiration against heat disturbances.

MATERIALS AND METHODS

Experimental site

The study was undertaken in an ongoing long-term fertilizer experiment (LTFE) started in the year 1972–73 at Birsa Agricultural University, Ranchi, India. The research field is situated at longitude of 85° 19' E and latitude of 23° 17' N, about 625 m above msl (mean sea level) and experiencing hot moist sub-humid climate.

Treatment details

The experiment was laid out in the year of 1972 under soybean (*Glycine max*) – potato (*Solanum tuberosum*) – wheat (*Triticum aestivum*) system and it was continued till 1983–84, and there after cropping system was changed to soybean-toria (*Brassica campestris*)–wheat until 1984–85 and then onwards soybean-wheat cropping system from 1986–87. The ongoing treatments of the LTFE, consisted of 10 fertilizer treatments, out of which only seven contrasting treatments were selected for this study; control (no fertilizer, no manure), 100% N, 100% NP, 100% NPK, 100% NPK + hand weeding (100% NPK+HW), 100% NPK+FYM and 100% NPK + lime. The experiment was laid out with four replications in a randomized block design (RBD) having 100 m² individual plot size. Since 1972, control plots were without fertilizer. Recommended dose of fertilizers (100% NPK) was 25:13:33 kg NPK ha⁻¹ for soybean and 80:26:33 kg NPK/ ha⁻¹ for wheat

crops. Nitrogen, phosphorus and potassium nutrient were supplied through urea, diammonium phosphate, and muriate of potash respectively. In FYM treatment, FYM was applied at 10 t FYM ha⁻¹ during *Kharif* season of every year, while in treatment 100% NPK + lime, lime was applied in the *Kharif* season of every year at 0.40 t ha⁻¹. Based on last five years observation, average C (35%), N (1.4%), P (0.32%), S (0.2%), and Ca (0.5%) content in FYM were obtained. Pre-emergence (Imazethapyr) and post-emergence weedicide (2,4-D) was applied as per standard protocol in both soybean and wheat crops in all the treatments except in 100% NPK+HW and control where hand weeding was performed for controlling the weed during crop growth period.

Composite soil samples from 0–15 cm depth were collected from each plot at the pod formation stage of soybean (August, 2018) crop in soybean–wheat system. The gravimetric moisture content of soil was immediately determined. Substrate induced respiration (SIR) was measured by observing CO₂ evolution rate during the incubation of soil, which was mixed with dry powder of *Sesbania bispinosa* plant (leaves + tender stems) (C: N ratio 15.4) in a closed system. CO₂ trapped in NaOH (0.1N) was titrated with 0.1N HCl (Griffith *et al.* 2005). SIR rate calculated based on following equation:

$$mg \text{ CO}_2 \text{ g/soil/h} = \frac{(B - V) (N * E) * 1000}{DW * T}$$

Where, B = volume of standard blank HCl used for sample (ml); V= volume of standard HCl used for blank (ml); N = normality of HCl; E = equivalent weight of C in CO₂(E=22); DW = weight of dry soil (g); T = time of incubation in hrs.

The cumulative substrate induced respiration rate were also calculated after 96 hours by adding the substrate induced respiration rate at different interval viz. 24, 48 and 96 hours.

For heat stress samples were kept at 48 °C temperature for 24 hours and particular temperature (48 °C) was chosen because in the study area facing very hot summer (May–June), which is very frequent under changing climate scenario. After stress samples were placed in BOD incubator at 28 ± 2 °C for estimating the changes in above said properties at 1 and 30 days after heat stress.

Development of Resistance index (RS) and Resilience index (RL)

The resistance and resilience index were determined as described by (Orwin and Wardle, 2004): Resistance (RS) index was calculated the following equation

$$RS \text{ at } t_0 = 1 - \frac{2 |D_0|}{(C_0 + |D_0|)}$$

Where D_0 is the difference between the control (C_0) and the disturbed soil (P_0) at the end of disturbance (t_0).

This index was standardized by the control, as this takes into account differences in the amount of change that a disturbance could cause. The index for resilience (RL) was calculated as the following equation (Orwin and Wardle, 2004):

$$RL \text{ at } t_x = \frac{2 |D_0|}{(|D_0| + |D_x|)} - 1$$

Where D_x is the difference between control (C_x) and disturbed soil (P_x) at the end of disturbance (t_0). This index is standardized by the amount of change initially caused by the disturbance (D_0) as this determines the state from which it has to recover. The resistance and resilience indices ranged between -1 and +1.

Statistical analysis

All the data were analyzed using procedure proposed by Gomez and Gomez, (1984) for randomized block design of the experiment. Significance referred to in the results was $P < 0.05$. For calculation of analysis of variance (ANOVA) and Tukey honest significant different test (Tukey HSD), SAS software was used.

RESULTS AND DISCUSSION

Substrate induced respiration (SIR)

Long-term fertilization and liming had significant effect on substrate induced soil respiration (Table 1). In freshly collected soil (0 DAS), NPKL (100% NPK+ lime) plots showed highest CO_2 evolution rate (10.7 mg CO_2 g/soil/h) whereas NP plots (8.9mg CO_2 g/soil/h) recorded least SIR rate during first 24 hours and it was statistically at par with the control. NPKL plots had ~ 21% higher SIR rate than NPK plots, indicating the role of liming in substrate induced

soil respiration during initial 24 hours. The least value of SIR in NP plots indicated the adverse effects of imbalanced fertilization (Kumar *et al.*, 2014). Application of only nitrogenous or imbalance fertilization may change the microbial communities, which increased susceptibility to heat stress and decreasing SIR. Increasing trend in SIR value was recorded from 24 to 48 hours as compared to 0 to 24 hours in all the treatments except control and NPKL, although the rate of increment and/or decrement was very less with respect to treatments. During 24 to 48 hours, maximum SIR value was recorded in NPKF plots followed by NPK = NPKL = NP > control plots. During this period, NPKF plots recorded significantly highest soil respiration rate over rest of the treatments, whereas NP, NPK, NPKL had statistically similar SIR rate although it was higher than control plots. It showed that liming was not effective in enhancing SIR rate after certain period of time (24 hours). As time passes (48- 96 hours), the SIR value decreased drastically in all the treatments although the rate of decrement varied among the treatments. Among the treatments, NPKF recorded highest SIR value followed by NPKL = NPK > NP > control plots. The mean respiration rate during 48 to 96 hours decreased by 2.61 times compared to 24 to 48 hours (9.86 mg CO_2 g/soil/h). The FYM application probably sustains a microbial community that is able to respond quickly to the added substrate. Further, it can be stated that repeated application of FYM may enhance not only microbial diversity, stability of community structure and higher substrate utilization ability, but also soil functional stability (Griffiths *et al.* 2008).

When the soil was subjected to heat stress of (48 °C for 24 h), the effect of heat stress was significant in all the treatments. The SIR values after 24 hours of incubation was found highest in NPKL (11.5 mg CO_2 g/soil/h) plots and the least in NPK treatments (9.9 mg CO_2 g/soil/h). Under heat stress after 48 hours of incubation, CO_2 evolution rate showed a decline in all the treatments as compared to 24 hours of incubation (1 DAS). It is to be noted that heat stress at 48 hours of incubation (1 DAS) changed the trend as it was observed in treatments when there was no heat stress under same experimental condition. At 48 hours of incubation, SIR values in NP, NPK and NPKL plots were at par with each other. However, after

96 hours of incubation (1 DAS), no significant difference was observed. At 1 DAS, SIR value of all the treatments were less because one day is not sufficient to increase the proportion of tolerant or inactivated microorganisms (spores or dormant state) to induce an adaptation of microorganisms at the colony scale (Islam *et al.* 2019). At 30 DAS, CO₂ evolutions rate showed a decline starting from 24 hours incubation to 96 hours of incubation (table 1). At 24 hours after incubation (30 DAS) control plots had a highest SIR value (11.7 mg CO₂ g/soil/ h) whereas for

rest of the treatments a non-significant value was observed. At 48 hours of incubation, highest SIR value was observed in NPK plots, however, the value was at par with NPKF treatments and the least amount of CO₂ evolution was observed under control plot which was ~ 13% lower than NPK treated plots. After 96 hours of incubation (30 DAS) highest CO₂ evolution rate was found in control plots (4.28) and the least in NPKL plots, however, for rest of the treatments SIR value was at par with each other.

Table 1: Impact of long-term fertilization and heat stress on substrate induced soil respiration (mg CO₂ g/soil/ h)

Treatments	0 DAS			1 DAS			30 DAS		
	24 hrs	48 hrs	96 hrs	24 hrs	48 hrs	96 hrs	24 hrs	48 hrs	96 hrs
Control	9.07 ^D	8.97 ^C	2.79 ^D	11.3 ^{AB}	4.71 ^C	3.82	11.7 ^A	4.73 ^C	4.28 ^A
NP	8.93 ^D	9.93 ^B	3.68 ^C	10.8 ^B	5.64 ^B	3.72	10.2 ^B	5.33 ^{AB}	3.83 ^B
NPK	9.83 ^C	10.00 ^B	3.97 ^B	9.9 ^C	5.63 ^B	4.04	10.0 ^B	5.55 ^A	3.87 ^B
NPKF	10.30 ^B	10.47 ^A	4.38 ^A	11.1 ^{AB}	5.94 ^A	3.76	10.5 ^B	5.43 ^A	3.78 ^B
NPKL	10.78 ^A	9.93 ^B	4.04 ^B	11.5 ^A	5.58 ^B	3.85	10.5 ^B	4.97 ^{BC}	2.88 ^C
Mean	9.78	9.86	3.77	10.9	5.50	3.84	10.64	5.20	3.73
SE(d)±	0.13	0.02	0.06	0.2	0.05	0.20	0.2	0.10	0.09

0 D, 1 DAS and 30 DAS indicates fresh soil after sampling and days after heat stress, respectively. NPKF, NPK + farmyard manure; NPKL, NPK + Lime; hrs, hours. Means followed by same letter are not significantly different according to Tukey's honest significant difference test (at $P < 0.05$)

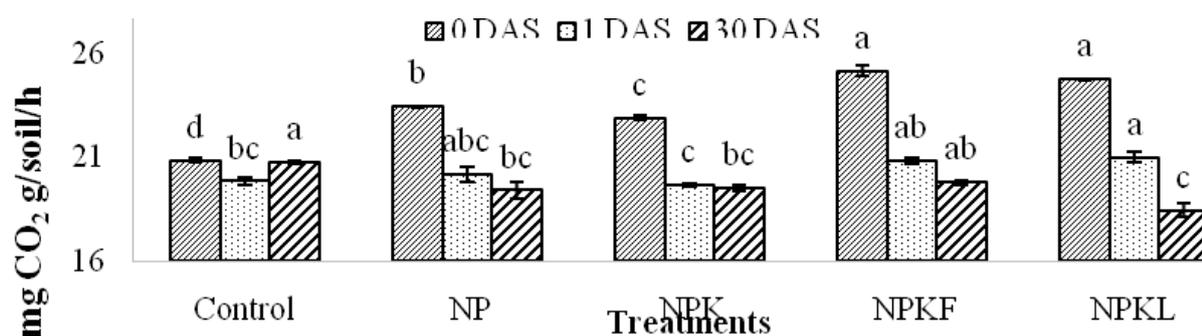
Cumulative respiration rate

Heat stress drastically reduced cumulative respiration rate in all the treatments although the reduction was less in control as compared to rest of the treatments (figure 1). This may be ascribed to different type of stress faced by control treatments from last five decades. Secondly, continuous adoption of without fertilization may lead to shift in microbial community structure and most probably developed groups of microorganism those have ability to resist any type of biotic stress. In fresh soil samples treatments, NPKF recorded significantly highest cumulative soil respiration rate over unbalanced and without fertilize treatments. Application of FYM may add readymade food to inherited microbes residing in soil as well add some groups of microorganism in soil, which act as an additive precursors to increase the number of active microorganism in soil. At 1 DAS, cumulative soil respiration rate ranged from 19.82 to 21.8 mg CO₂ g/soil/ h and no significant difference was found among treatments. In contrast to fresh soil samples, cumulative soil respiration rate was highest in

control and lowest in NPKL treatments, although no significant difference was recorded among treatments. Application of lime along with balanced fertilization increased the bacterial population by increasing the pH of acidic Alfisol (Vishwanath *et al.*, 2020), the bacterial population is known to be offer less resistance under any abiotic stress (Kumar *et al.* 2013) that led to less SIR under NPKL treatment. In nutshell, heat stress had performed negative impact on cumulative SIR rate and even 30 DAS the cumulative SIR rate was not removed to its initial value.

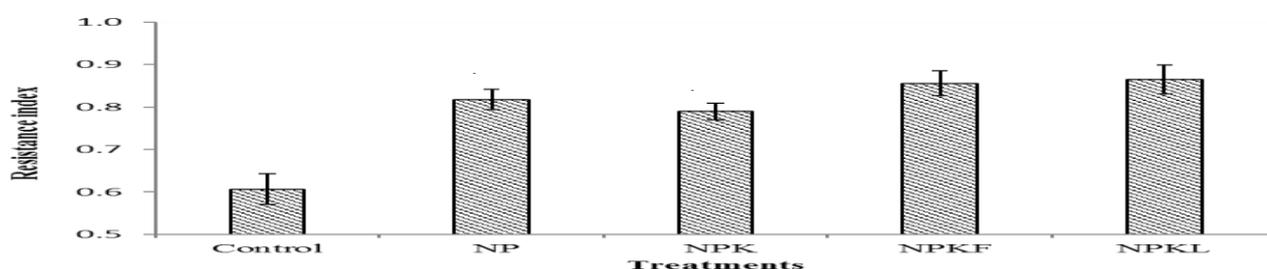
Table 2: Resilience index of substrate induced soil respiration at different time interval after 30 days of stress

Treatments	24 hrs	48 hrs	96 hrs
Control	-0.09 ^B	0.00 ^A	-0.19
NP	0.31 ^{AB}	-0.03 ^{ABC}	0.17
NPK	-0.04 ^B	-0.01 ^{AB}	-0.35
NPKF	0.50 ^A	-0.05 ^{BC}	-0.02
NPKL	0.52 ^A	-0.06 ^C	-0.72
Mean	0.24	-0.03	-0.22
SE(d)±	0.15	0.01	0.28



❖ Bars indicate standard errors of mean

Figure 1: Cumulative substrate induced soil respiration after heat stress under long term fertilization experiment



❖ Bars indicate standard errors of mean.

Figure 2: Resistance index of substrate induced soil respiration after heat stress.

Resistance and resilience index

Resistance is the ability of a soil system or process to stay unchanged in the face of a particular abiotic stresses/disturbance (Griffiths *et al.* 2013). The data on resistance index (figure 2) revealed that NPKF = NPKL recorded highest resistance (0.86) against heat stress followed by NP (0.82) > NPK (0.79) > control (0.61). FYM application increases soil organic matters, which in turn increased the sustainability of soil microbial activity to retain its function, making it more resistance to abiotic stress. The high SIR resistance index of FYM / lime in soil can also due to presence of more diverse group of micro-organisms, which probably influenced by the quantity as well as quality of the organic matter and other cultural practices favoring substrate utilization in these soils (Kumar *et al.* 2014). From the data on resilience index (table 2), it was noticed that after 24 hours of incubation (30 DAS), resilience index of NPKL (0.52) and NPKF (0.50) being at par with each other, was superior to all other treatments, the least resilience index was found in NPK and control plots. However, after 48 hours of incubation, highest resilience index was

found in control plots (0.0) whereas lowest value was observed in NPKL plots (-0.06). At 96 hours of incubation, the value of resilience index was non-significant among the treatments. The higher value of SIR at 30 DAS than undisturbed soil lead to either higher value of resilience index (RL) or negative value of resilience on 30 DAS. This might be due to higher flux of carbon dioxide after stresses, which could have been related to higher energy demand for the restoration of injury caused to the population (Braun *et al.* 2010).

From this study, it may be concluded that substrate-induced respiration (SIR) and their resistance and resilience against heat stress were strongly affected by different long-term fertilization treatments. The highest resistance and resilience in the treatments NPKF/NPKL indicate the presence of microbial functional communities/diversity, which were resistant and resilient to heat stress. Applying unbalanced fertilizers (NP) may be weakening the resistance and resilience of soil functions; thus, addition lime/FYM along with balanced fertilizers is necessary for the stability of soil functions (soil respiration) against abiotic stress in a long run.

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