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Assessment of soil resilience to abiotic stress under diverse management regimes

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ABSTRACT

Agriculture is considered to be one of the most vulnerable sectors to diverse stresses. Abiotic stresses such as heat, drought, salinity, heavy metal toxicity etc., limit crop productivity. Soil management plays crucial role in sustaining productivity under multiple abiotic stresses. It is globally agreed that effective resource management restores natural resources and combats stresses. With this view, soils from a 13 years old long-term integrated nutrient management (INM) experiment with diverse management strategies were selected and exposed to abiotic stresses viz. heat, copper toxicity and chloroform fumigation in an incubation experiment. Activities of few hydrolytic enzymes as bioindicators and their resilience towards different stressors under the influence of INM practices were studied to identify the best INM practise to manage abiotic stresses. Results revealed that soils applied with FYM/Green manure had enhanced resilience against different stressor than the sole Chemical fertilized soil. The INM comprising of ½N+½P+K of soil test crop response (STCR) based nutrients [Soil test based fertilizer dose -125:28:62 (for kharif) and 140:40:80 (for rabi)] with FYM (@7.5 tons/ha) and biofertilizers [Azospirillum + Phosphobacter (@4.0 kg/ha)] maintained highest soil enzymatic activities when exposed to different abiotic stresses The INM treatment enhanced the alkaline phosphatase (15.87%, 17.31%, 15.48%), acid phosphatase (25.46%, 18.06%, 27.15%), β-glucosidase (27.3%, 29%, 69.18%) and urease (160.42%, 133 %, 129.41%) activities over the conventional farming when exposed to heat stress, copper toxicity and chloroform fumigation respectively. Integration of nutrients restored the soils and exhibited less stressed when exposed to abiotic stresses, as evidenced by lowest metabolic quotient (qCO_2) of 18.43 $\mu g\ CO_2\text{-}C\ h^{-1}\ \mu g^{-1}$ MBC. Results also established that with the progress of incubation, INM practices as mentioned earlier, gained speediest recovery of enzyme activities from the different stresses. Among INM, treatment comprising of (½N+½P +K) of STCR-based nutrients + FYM+ biofertilizers triggered the biological indicators when exposed to different abiotic stresses to exert more resistance to alleviate the impact of stress. Therefore, it can be recommended as the best management option for combating abiotic stress after field experiments in diverse agro-ecological condition.

1. Introduction

Agriculture is vulnerable to abiotic stresses such as salinity, heat, drought, nutrient deficiency or toxicity, heavy metals/metalloids and flooding limit crop quality and productivity world-wide. However, this situation becomes more problematic in developing countries like India, where they cause food and nutritional insecurity for large populations and poverty, particularly in rural areas. Drought has affected about 33% of the arable land and a further 35% is prone to such damage. While salinity, acidity and nutrient deficiency / toxicity render more than 100 m ha of land uncultivable. By 2050, 51% of wheat growing areas of IGPs will be under heat- stressed area (Ortiz et al., 2008). In this scenario, it is widely urged that strategies should be adopted that may be used to get maximum crop stand and economic returns from stressful environments. Soil management can

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play an important role for sustaining crop productivity under multiple abiotic stresses (Brussard et al., 2007). Resource management can restore natural resources and combats the stresses, stabilizes the ecosystem and optimize crop by improving soil health. Integrated nutrient management (INM), in this context, pays significant contribution. Under INM, the production system as a whole acquires resistance to extreme events either by vitalizing the soil ecosystem with rich biodiversity, elaborating enzymes and metabolites, particularly, the mycorrhizal diversity and their life saving functioning or by triggering important metabolic activities of plant (Evelin et al., 2009) or by both. Building healthy soil is a crucial element in helping farms to cope with stresses. In INM, comprising of all possible sources of nutrient including rational use of chemical fertilizers in conjunction with composts, manure help in soil aggregate and microhabitat, reducing soil moisture loss protecting soils from erosion and enrich the soil with organic matter, respectively, to increase WHC, make nutrients more accessible to the plant and more carbon sequestration (Tirado and Cotter, 2010). The plant growth promoters (PGP) microbes contain useful variation for tolerating abiotic stresses like extremes of temperature, pH, salinity and drought; heavy metal and pesticide pollution (Ali et al., 2009,). Inclusion of such tolerant PGP microbes in INM is expected to offer enhanced plant growth and yield even under a combination of stresses.

The response of an ecosystem to any stress has two components, resistance and resilience, whose combined effects determine what ecologists refer to as "ecosystem stability". Resistance is the inherent capacity of the system to withstand disturbance, whereas resilience is the capacity to recover after disturbance. Microbial community structure and soil specific functions, in general, enzymes activities, in particular are very sensitive to stress (Giller et al., 1998; Griffiths et al., 2000; Sardans and Penuelas, 2005). They provide information well in advance with meaningful manifestation through their activities and preponderance. Thus, they are useful tools for studying stress resistance capacity of soil under different managements.

Extensive research has been carried out on occurrence and functional diversity of agriculturally important microbes in stressed environments as reviewed by several authors (Zahran 1999; Venkateswarlu et al. 2008). Use of these microorganisms *per se* can alleviate stresses in crop plants thus opening a new and emerging application in agriculture. Assessment of resistance capacity through estimation of biological indicators under long-term INM systems for identifying most efficient management to withstand the probable upcoming stress is a new approach. Thus, the present study hypothesised that i) Soils under organic- input- based farming and integrated nutrient management system are more resistant ii) Whereas conventional farming is less resistant under same stress events iii)The more resistant the soil the more healthy the land is. With this background, to test those hypothesises , the present study aimed to 1) to assess resistance capacity of the different management strategies towards different stressors by studying differential changes of indicator's behaviour and 2) identify the best management practice to resist probable upcoming stresses for sustainable production.

2. Material and methods

2.1 Site description:

A long-term experiment on integrated nutrient management (INM) of 13 years old in Central Research Farm, Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya was selected, which is situated at 23˚N latitude and 89˚E longitude at an elevation of 9.75m above mean sea level. The long term INM experiment consists of the following management practices: 1. No fertilizer 2. Uncultivated fallow 3. Conventional farming [Soil test based fertilizer dose - 125:28:62 (for kharif) and 140:40:80 (for rabi)] 4. INM [½N + P + K] of T_3 + Green manuring (GM @2.5 tons/ha) 5. INM [½N + ½P + K] of T_3 + Green manuring (GM @2.5 t/ha) + [*Azospirillum* + Phosphobacter (@4.0 kg/ha)] 6. INM [½N + ½P + K] of T_3 + FYM (@7.5 tons /ha) + [*Azospirillum* + Phosphobacter (@4.0 kg/ha)] 7. Organic input-based farming (*Azospirillum* + Phosphobacter (@4.0 kg/ha) + FYM (10.5 tons/ha).

2.2 Collection and preparation of soil sample

Soils were collected 7-10 days after the harvest of *boro* rice from the seven different experimental plots having different management practices following standard protocol with the premise that in absence of any standing crop soil internal system will be stabilized. Each treatment plot consists of 5×4.5 m in size, and soils were collected from the plough layer (0 to 15 cm) randomly from different points. After drying and quartering, soils were sieved with 2 mm nylon sieve to obtain homogenized 2 mm- sized aggregates. Finally representative soil samples were stored in dry polythene bags for the incubation study. A portion of live soil was store at 4ºC for microbial and biochemical studies.

2.3 Treatment details

Four 700g of air dried soil from each representative soil samples were weighed and placed in respective 1 L plastic beaker. Three replications for each sample were taken. This provided 84 experimental units. The soil water content was adjusted to 60% of the water holding capacity (WHC) and the water content was maintained by applying water lost during incubation by maintaining constant weight of experimental units. Samples were pre-incubated for 4 days at 25˚C in a dark room before different stressor disturbance application. After the pre-incubation period, the following treatments were administered: (1) control (C) no stress imposition, (2) soil subject to 50 ˚C for 3 hours, (3) amended with copper (powdered $CuSO_4$ to give 500μg Cu g^{-1} dry soil) and (4) exposed to chloroform fumigation for 3 hours. After exposure of samples to stressors, they were incubated at 25˚C for 105 days and the samples were watered from time to time in order to maintain 60% of the water holding capacity (WHC) by periodical weighing of the beaker. The beakers were arranged following factorial split completely randomized design with three replications

2.4 Sampling procedure and method of analysis:

Substrate-induced respiration (SIR) was estimated following the method developed by Anderson and Domsch (1978) to provide a quick estimation of living microorganism carbon biomass microbial activities in soils. After imposition of stress 100g soils was placed in L conical flask and amended with 0.1% glucose solution. 60% WHC of experimental soil was maintained although the experimental period. CO_2 released by microbial respiration was trapped in standard alkali and unspent alkali was back titrated with standard HCL and phenolphthalein as an indicator to estimate the CO_2 evolved from soil. Readings were taken after every 24 hours interval for 7days followed by 48 hours interval for 6 days and after every 72 hours interval for next 9 days and continued with 1 week intervals for 21 days. Microbial biomass carbon (MBC) was determined by chloroform fumigation and extraction method (Joergenson, 1996).

During the whole incubation period, soil samples were withdrawn 6 times at an interval of 21 days for the estimation of enzymatic activity viz. phosphatase (alkaline and acid), β- glucosidase, and urease activity. Phosphatase (acid and alkaline) and β- glucosidase activity was determined by estimation of *p*-Nitrophenol released after the incubation of soil with *p*-Nitrophenol phosphate and *p*-Nitrophenol glucoside for 1 hr at 37˚ C (Tabatabi and Bremner, 1969, Evazi and Tabatabai, 1988). Urease activity of soil was determined by estimating the amount of ammonia released after the incubation of soil samples with urea solution for 2 hours at 37˚ C (Tabatabai and Bremner, 1972).

2.5 Statistical analysis:

The effect of different management and stress regime on soil biological indicators microbial communities and their functions was analysed statistically by ANOVA and software SPSS (17.0).

3. Result and discussion

3.1 Substrate induced respiration (SIR)

In general, irrespective of stress after 42 days of incubation, fallow soil (T_2) had significantly higher CO_2 evolution over no fertilized soils (T_1) by about 25%. The relatively undisturbed microhabitats prevailed under fallow secured the microbial population to environmental stress imposed on soil. Shifts in microbial communities under fallow, thus, restored, and utilized the resources efficiently even under stressful environment. INM option, (½ N + ½ P + K) of STCR based fertilizer + FYM + biofertilizers (BF), T_6 and organic input-based farming comprising of BF+FYM, T_7 soil showed significant increase in CO_2 production by about 22% and 19.6% respectively, over T_1. On the other hand, conventional farming with STCR based fertilizer dose, (T_3) recorded only 6.15% increase in CO_2 production than T_1. FYM integration along with chemical fertilizers and biofertilizers under T_6 probably supplied sufficient organic carbon and balanced nutrition to the existing communities to respire efficiently. In addition, repeated application of FYM for last 13 years in the experimental plots might establish stable communities which resist the abiotic stresses and performed their activities in soil. This confirms the earlier results of Katayama et al. (1998) who revealed that long-term application of FYM to soil resulted in development of more diverse microbial community and establishment of stable community. Substrate utilization ability of soil was enhanced by the repeated application of FYM (Marwati et al., 2003; Toyota and Kuninaga, 2006). Greater microbial biomass conserved in FYM-based nutrient management resulted in higher respiration. Green manuring-based nutrient management, on the other hand, failed to perpetuate respiration rate as that of FYM-based nutrient management. This is due to less residual effect of green manure because of its perishable nature. Thus, Green manuring-based nutrient management harboured less resistant microorganisms against different stresses.

3.2 Microbial biomass carbon (MBC)

Among the different treatment combinations, INM (½ N + ½ P + K) of STCR based fertilizer+FYM+BF, T_6 maintained significantly higher MBC (35.68%) followed by organic input-based farming comprising of BF+FYM, T_7 (31.42%) and INM (½N + ½P + K) of STCR base fertilizer+GM+BF, T_5 (16.25%) than T_1. Higher soil MBC reflects the degree of C immobilization, and higher concentrations indicate more C assimilation into microbial biomass, which reduces the losses of C through chemical and physical processes (Liu et al. 2012). On the basis of this argument T_6 may be considered as the most resistant management to combat abiotic stresses. The microbial biomass is increased by management practices that increase inputs of organic carbon to soil and improve the chemical and physical conditions experienced by microorganisms in soil. This corroborates the finding of Nath et al. (2011). Toyota and Kuninaga (2006) also reported that functional stability was increased by repeated application of cow manure and coffee extraction residue composts. It is interesting to note that in spite of higher FYM incorporated in T_7, lower MBC was recorded under this management practice as compared to T_6. This might be due to reduced nutrients for microbial assimilation leading their growth and development.

3.3 Microbial metabolic quotient (qCO_2)

Microbial metabolic quotient (qCO_2) of glucose amended (0.05%) soils under different management strategies were measured by C_{CO2}/C_{mic} following the method suggested by Anderson, 2003 (Table 1). Results showed that soils of different management responded different qCO_2 value under the influence of abiotic stresses. Fallow soil, T_2 exerted least qCO_2 followed by INM (½N + ½P + K) of T_3 +FYM+BF, T_6; organic input-based farming comprising of BF+FYM, T_7 and INM (½N + ½P + K) of T_3 +GM+BF, T_5. Higher MBC and relatively lower respiration as observed in present investigation favoured lower qCO_2. The lower qCO_2 under fallow indicates that this soil is under low stress condition. Due to less perturbation in fallow soil, microbes were under protected habitat. Thus, normal respiration was not hampered. Among the integrated nutrient management practices least qCO_2 was attended by treatment consisting of (½N + ½P + K) of T_3 +FYM+BF, T_6. In this context, T_6 exerted highest resistance to stress. STCR-based nutrient treatment, T_3 exhibited highest qCO_2 all-through the incubation period which indicates that microbes under this management were highly in physiologically disturbed condition. To resist the stress microbes respire more and more to get energy under stress condition resulting higher qCO_2. Elevated qCO2 under stressed soil was also noticed by Filser et al. (1995) and Giller et al. (1998). This increased microbial qCO2 under stress condition is due to the diversion of more energy into maintenance as compared to reproduction as suggested by Killham (1985). In INM systems due to the improvement in soil structure, balanced nutrition and available moisture, microbes are in less stressed condition which resulted efficient microbial utilization of carbon and least qCO2 (Sharma et al., 2015).

3.4 Alkaline phosphatase activity (AlP)

Under no stress condition, the INM options, (½ N + ½ P + K) of STCR based fertilizer in conjunction with FYM and biofertilizer (T_6) maintained significantly the highest level (290.4 µg PNP $g^{-1}h^{-1}$) of alkaline phosphatase activity (AlP) of soil followed by organic input based farming comprising of FYM and biofertilizers, T_7 (283.3 µg PNP $g^{-1}h^{-1}$), and fallowing, T_2 (278.6 µg PNP $g^{-1}h^{-1}$).

When exposed to heat stress, fallow soil, T_2 maintained significantly highest AlP activity followed by INM (½N + ½P + K) of T_3 +FYM+BF, T_6; organic input-based farming comprising of BF+FYM, T_7 and INM (½N + ½P + K) of T_3 +GM+BF, T_5. Least alkaline phosphatase activity was detected under STCR based nutrient management practice (T_3) and control soil with no fertilizer (T_1). Secured habitat under undisrupted fallow might provide assured resource for carrying out necessary AlP activity by the existing microorganisms in soil. Nutrient integration as a whole improved the AlP activity under heat stress over T_1 (Fig. 1). Among the nutrient integration the least AlP activity was recorded under the influence of green manuring on conjoint application of chemical fertilizer only (T_4). The results highlighted a constant difference among different managements over time. Inhibitory effect of heat stress on AlP activity under different management was also detected during 105 days of incubation. However, the extent of inhibition and its subsequent recovery all-through the incubation period were different under different management practices (Fig. 1). Fallow soil subjected to heat stress lasted for 21 days and thereafter AlP recovered its activity progressively till the end of the incubation. Disturbance duration was longest in case of conventional farming with STCR based fertilizer recommendation i.e. up to 84th of

Table1. Substrate induce respiration (SIR, cumulative CO_2-C in µg g^{-1} h^{-1} for 42 days), microbial biomass carbon (MBC, µg/g) and microbial metabolic quotient (qCO_2, µg CO_2-C h^{-1} µg^{-1} MBC) of soils under different management practices as influenced by different abiotic stresses

Nutrient managements	SIR	MBC	qCO_2
T1	7491.0^{e}	5954.6^{f}	20.67^{a}
T2	9344.0^{a}	8171.3^{a}	18.53^{ef}
T3	7952.0^{d}	6285.8^{e}	20.20^{b}
T4	8178.0^{cd}	6677.3^{d}	19.95^{c}
T5	8349.0^{c}	6922.7^{c}	19.64^{d}
T6	9146.0^{ab}	8079.6^{a}	18.43^{f}
T7	8960.0^{b}	7825.8^{b}	18.65^{e}

* The values present the cumulative of means for 16 observations during 42 days of incubation. Values in the same column followed by same superscript are not significantly different (p<0.05) and different superscript indicates significant difference (P<0.05) among the values by Duncan's Multiple Range Test.

incubation period. Under green manuring treated soils (T_4 and T_5), such inhibition was restricted up to 63th days of incubation. Waldrop and Finestone, 2004 reported similar reduction in enzyme activities for several weeks or months in soil temperature incubation experiments. However, FYM treated soils, T_6 and T_7 checked further reduction of alkaline phosphatase activities beyond 42 days of incubation over conventional farming. This could be explained by the fact that FYM treated soils are often dominated by bacteria in agricultural soils (Fierer et al., 2005) and are more tolerant to heat (Frey et al., 2008 ; Keiblinger et al., 2012). Heat stress might lead to change in microbial community composition and diversity creating a new microbial community structure that is particularly well adapted to heat stress under INM systems and has significant functional stability (Grivan et al., 2005 and Schimel et al., 2007). As reported microorganisms under INM are more heat resistant and often produce thermo-stable enzymes (Trosvik et al., 2002). Regarding recovery, fallow soil attained the speediest rate (10.8% recovery) followed by INM comprising of mineral fertilizer, FYM, and biofertilizers, T_6 (9.9%) and organic input based farming, T_7 (9.4%) respectively.

Nutrient integration as a whole succeeded in maintaining higher AlP activity under copper toxicity over control (Fig. 1). INM option comprising of (½N + ½P + K) of T_3 +FYM+BF, T_6 supported 21.8% increased AlP activity over T_1 followed by organic input-based farming, T_7 i.e. achieving 19.7% increment over T_1. Among INM, the least increment in AlP activity over T_1 was attained by integration of chemical fertilizer with green manure, T_4. Whereas, conventional farming, T_3 recorded only 3.8% increment over T_1. Results showed that in comparison to heat stress, the inhibitory effect of copper toxicity on alkaline phosphatase activity of different management soils lasted for longer duration during 105 days of incubation (Fig. 1). It was observed that T_2, T_6 and T_7 soil subjected to copper toxicity lasted for 63 days and thereafter AlP activity recovered its activity progressively till the end of the incubation. Whereas, disturbance duration lasted up to 105 days in case of T_1, T_3 and T_4 and thus, no recovery was recorded. Among INM, T_6 soil attained the speediest rate (9.1%) followed by T_7 (7.9%) respectively. Resistance and resilience are strongly affected by soil Cu^{2+} and pH (Lock and Janssen, 2003). Creation of Cu toxicity by addition of $CuSO_4$ probably increased Cu^{2+} and declined pH which strongly affected the resistance of AlP activity, particularly, under STCR-based nutrient management system. On the other hand, INM systems generally arrested such effect to a greater extent. FYM-based nutrient management practices, in this regard, exerted higher resistance. Occlusion of Cu^{2+} with higher organic matter (Ma *et al*., 2006) in FYM amended soil and immobilization of free Cu^{2+} by the production of exo-polymers (Kunito et al., 2001) of diverse microorganisms under INM systems reduced Cu^{2+}. Thus, reducing Cu^{2+} bioavailability relieves the stress on microbial community elaborating AlP enzyme. Soil tolerance to copper stress as observed by Griffiths et al. (2005) was more suppressed in no organic matter amended soil than in the soil amended with sufficient organic matter. Therefore, our findings clearly demonstrated that repeated application of GM and FYM supported functional activity of AlP activity under Cu stress. This corroborates the earlier finding of Wada and Toyota (2007).

On exposure to chloroform fumigation, INM option comprising of (½N + ½P + K) of T_3 +FYM+BF, T_6 being at par with fallow, T_2 showed significantly highest AlP activity followed by organic based farming, T_7 and INM (½N + ½P + K) of T_3 +GM+BF, T_5. It is known that the physical structure of the soil influences the location and composition of the soil microbial community through pore size and distribution (Young and Ritz, 2000) and thus, soil structure may protect soil microorganisms from fumigation (Kuan et al., 2007). This may be the case because it is well known that organic amendment stimulates soil aggregation, typically expressed as higher MWD values (Wanger et al., 2007). In this study, FYM/Green manuring applied soils shows enhancement of resistance than in the chemically fertilized soil. Our finding is in same line with Dungan et al. (2003), who reported that organic matter amended reduced impact of fumigation of soil. Longest extent of inhibition of AlP activity subjected to chloroform was recorded in T_3 and T_1 i.e. 84 days of incubation. Integration of fertilizer with Green manuring (T_4 and T_5) cut the disturbance duration by 63 days. However, FYM based INM (T_6 and T_7) checked further reduction of alkaline phosphatase activities beyond 42 days of incubation over conventional farming (Fig. 1). This corroborates the earlier findings of Dungan et al. (2003) who found that microbial activities was decreased by soil fumigation and was suppressed throughout 12 weeks of incubation. Such activities were recovered when the soil was amended with 3% (w/w) manure. Griffiths et al. (2005) also found that soil resilience was enhanced in soil amended with digested and undigested sewage sludge compared to un-amended soils.

3.5 Acid phosphatase (AcP) activity

Results revealed that under no stress condition, INM (½N + ½P + K) of T_3+FYM+BF, T_6 being at par with fallow, T_2 maintained significantly the highest level of acid phosphatase (AcP) activity of soil followed by organic based farming, T_7; INM (½N + ½P + K) of T_3 +GM+BF, T_5 and INM (½N + P + K) of T_3 +GM, T_4. When exposed to heat stress, INM (½N + ½P + K) of T_3+FYM+BF, T_6 and organic based farming, T_7 recorded highest increment in AcP activity i.e. 34.8% and 32.9%

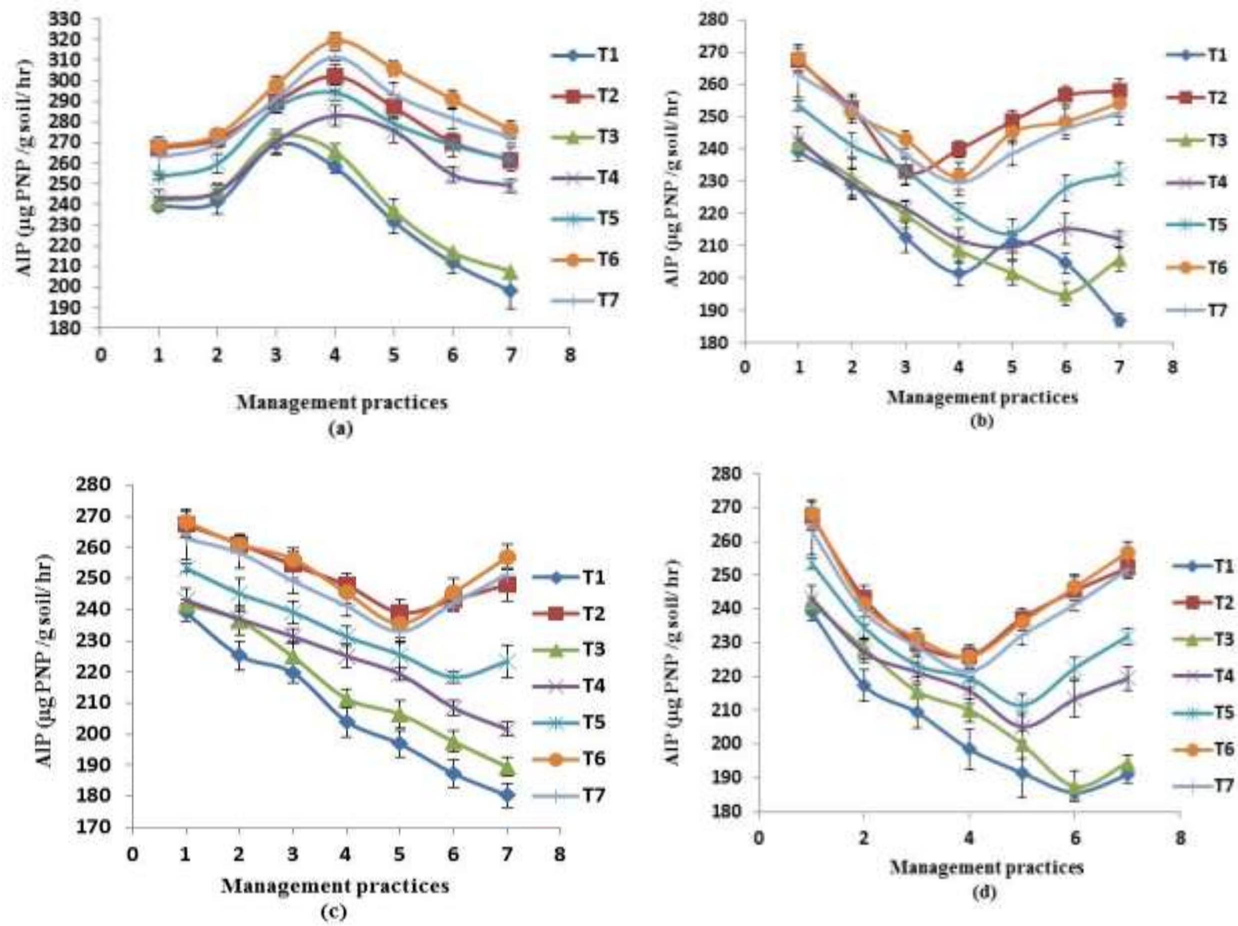

Figure 1. Trend of Alkaline phosphatase (AlP) activity (µg PNP /g soil/ hr) of soils under different management practices during 105 days of incubation as influenced by different abiotic stresses *viz.* (a) no stresses (b) heat stress (c) copper toxicity and (d) chloroform fumigation. Error Bars indicate standard deviation of mean.

respectively over T_1, whereas, T_3 recorded the least increment over T_1 (8.6%). In this context, FYM or GM treated soils showed superior impact on AcP activity to heat stress. This is confirmed by the findings of Katayama et al. (1998) who argued microbial community was more diverse in soils amended with the FYM. Katayama et al. (2002) further found that microbial community structure in the FYM amended soils was slightly changed by the addition of glucose and peptone, while that in solely chemical fertilized soil was greatly changed, and concluded that long-term application of FYM to soil resulted in the establishment of stable community. Thus, stable microbial communities and diversity in substrate utilization empowered the INM systems with higher resistance to heat stress. Results showed that disturbance duration created by heat lasted differently for AcP activity depending on the management strategy adopted (Fig. 2). T_2, T_6 and T_7 subjected to heat stress lasted for 42 days and thereafter acid phosphatase recovered its activity progressively till the end of the incubation. Inhibition of acid phosphatase activity was restricted to 63 days under treated soils T_1, T_3, T_4 and T_5 treated soils. Generally, longer the heat disturbance duration, greater will be the impact on acid phosphatase activity. Such recoveries of enzymes in soil amended with manure were highlighted by Katayama et al. (1998) and Dungan et al. (2003). Regarding recovery, T_7 attained the speediest rate (12.5%) followed by T_2 and T_6 (12.3 % and 7.7%) respectively.

Similarly, under copper toxicity, INM (½N + ½P + K) of T_3+FYM+BF, T_6 being at par with fallow, T_2 maintained significantly the highest level of AcP activity of soil followed by organic based farming, T_7; INM (½N + ½P + K) of T_3 +GM+BF, T_5 and INM (½N + P + K) of T_3 +GM, T_4. Soils of T_2, T_6 and T_7 subjected to copper toxicity lasted for 63 days and thereafter acid phosphatase recovered its activity progressively till the end of the incubation. However, in case of T_1, T_3, T_4 and T_5 inhibition of AcP activity were restricted to 84 days (Fig. 2). Thus results reveal that recovery of AcP activity of soil was faster in FYM treated soil in

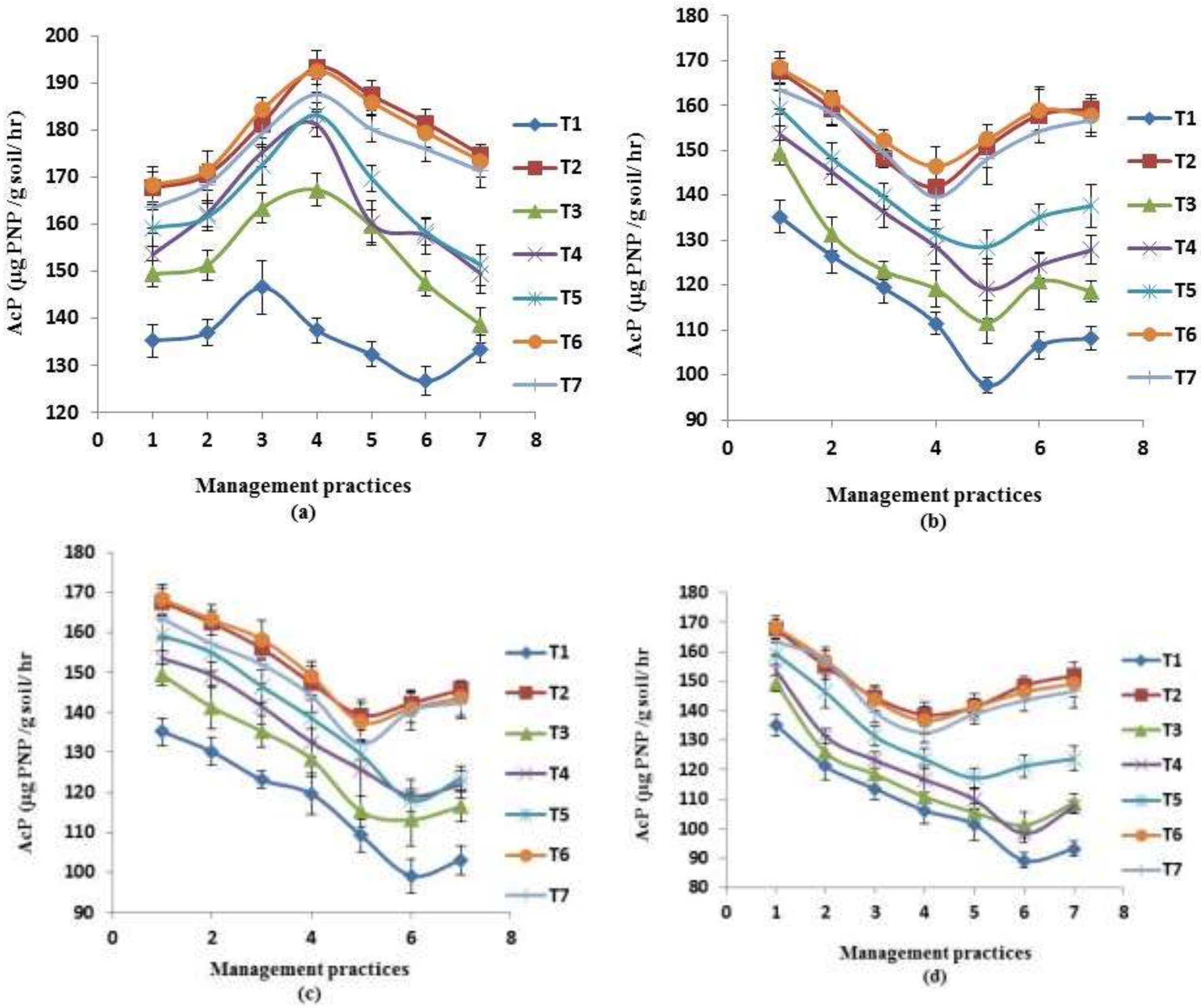

Figure 2. Trend of acid phosphatase (AcP) activity (µg PNP /g soil/ hr) of soils under different management practices during 105 days of incubation as influenced by different abiotic stresses *viz.* (a) no stresses (b) heat stress (c) copper toxicity and (d) chloroform fumigation. Error Bars indicate standard deviation of mean

comparison to other treatment options. Increased AcP activity to copper toxicity under FYM treated soils can be explained by two different ways: 1) diverse acid phosphatase microbial population under protected niche, 2) reduced bioavailability of Cu^{2+} ions. This phenomenon is explained in AlP activity to copper stress in earlier discussion.

When exposed to chloroform fumigation, INM (½N + ½P + K) of T_3+FYM+BF, T_6, in particular, recorded the highest increment in AcP activity followed by organic based farming, T_7 and INM (½N + ½P + K) of T_3 +GM+BF, T_5. Sole STCR base fertilizer treatment, T_3 recorded the least increment in AcP activity over T_1. Inhibitory effect of AcP activity of T_2, T_6 and T_7 soil subjected to chloroform fumigation lasted for 42 days and thereafter AcP activity recovered progressively till the end of the incubation. In case of T_5 the inhibition period lasted by 63 days and thereafter recovery started gradually. In case of T_1, T_3 and T_4 inhibition of acid phosphatase activity was restricted to 84 days. Thus results reveal that recovery of AcP activity of soil was faster in FYM treated soil in comparison to other management options (Fig. 2). Toyota and Kuninga (2006) in their experiment also established the role of repeated application of FYM on recovery of soil functions and biological activities.

3.6 β-glucosidase activity

Results revealed that under no stress condition, INM (½N + ½P + K) of T_3 +FYM+BF, T_6 was at par with fallow,T_2 and show significantly the highest level of β-glucosidase activity of soil followed by organic based farming of FYM+BF, T_7; INM (½N + ½P + K) of T_3 +GM+BF, T_5 and INM (½N + P + K) of T_3 +GM, T_4 (Fig. 3). β-glucosidase activity of differently managed soil maintained the same trend when exposed to heat stress.Under heat stress, INM (½N + ½P + K) of T_3 +FYM+BF, T_6 (93.7.1%) and organic based farming of FYM+BF, T_7 (91.6%) recorded highest increment in β-glucosidase activity over T_1. whereas conventional farming, T_3 recorded the least increment of 17.9 % over T_1. Results showed relative efficacy of different management practices to heat stress in maintaining β-glucosidase activity in soils (Fig. 3). Management options either having GM and FYM application showed higher β-glucosidase activity than chemically fertilized soils. Substrate availability under the management options triggered β-glucosidase activity as this enzyme is considered the most predominant one for carbon transformation in soil. Disturbance duration on β-glucosidase activity created by heat lasted differently depending on the management strategy adopted (Fig. 3). T_2, T_6 and T_7 subjected to heat stress lasted upto 42th day and thereafter β-glucosidase recovered its activity progressively till the end of the incubation. β-glucosidase activity was inhibited for longer duration in T_4 and T_5 i.e. upto 63th day but thereafter recovery started. No recovery was recorded under T_1 and T_3 along the whole incubation period (Fig. 3). In other experiments, different authors also pointed out that only chemically fertilized soils did not improve soil functionalities under stressful environment (Griffith et al., 2005). Among different treatment combinations, INM (½N + ½P + K) of T_3 +FYM+BF, T_6 attained the speediest rate (19.1%) of recovery followed by organic farming, T_7 (16.4%). This event might be explained as discussed earlier under AlP activity to heat stress.

Under copper toxicity, integrated nutrient management as a whole improved β-glucosidase activity over soil where no fertilizer was applied. However, fallow,T_2, INM (½N + ½P + K) of T_3+FYM+BF,T_6 and organic farming, T_7 were statistically at par and recorded 89.8% increased β-glucosidase activity over no fertilize,T_1. STCR base fertilizer treatment, T_3 recorded only 14.5 % increased β-glucosidase activity over T_1. Results also inferred that

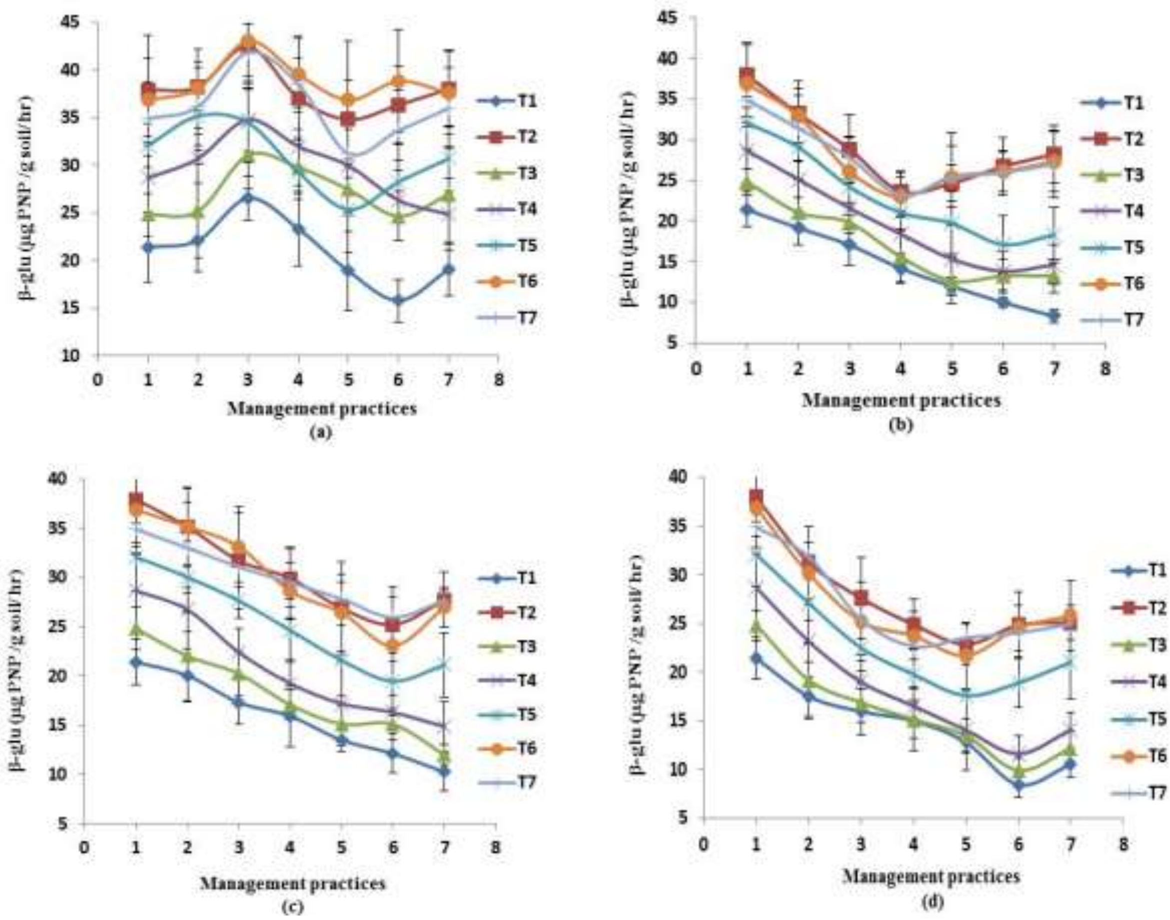

Figure 3. Trend of β-glucosidase (β-glu) activity (µg PNP /g soil/ hr) of soils under different management practices during 105 days of incubation as influenced by different abiotic stresses *viz.* (a) no stresses (b) heat stress (c) copper toxicity and (d) chloroform fumigation. Error Bars indicate standard deviation of mean.

disturbance duration created by copper toxicity on β-glucosidase activity lasted differently depending on the nature of management strategy adopted (Fig.3). Fallow,T_2; chemical fertilizer + GM + BF,T_5; chemical fertilizer + FYM + BF,T_6 and organic farming, T_7 subjected to copper toxicity lasted upto 84th day but a small recovery was seen in 105th day of incubation period. In case of soils of no fertilizer,T_1, conventional farming,T_3 and chemical fertilizer + GM, T_4 inhibition lasted up to 105th day. Relatively longer persistence of Cu^{2+} in copper contaminated soils under different managements might be the reasonable explanation of the long-lasting effect of copper. Inhibitory effect of copper was also reported by Sethi and Gupta (2015). However, results revealed that recovery of β-glucosidase activity of soil was faster in INM options in comparison to conventional farming. Under management options like no fertilizer, T_1 and STCR-based nutrient management, T_3 the intensity of copper toxicity to β-glucosidase activity was higher as compared to those of FY/GM based integrated nutrient management options. β-glucosidase as such is highly sensitive to copper (Geiger et al., 1998). Soil matrix is another important factor influencing the extracellular enzymes from copper toxicity. Organic colloids in soil matrix under INM options, particularly of FYM/GM treated plots specifically bind Cu^{2+} ions. The formation of ternary complexes between mineral surface, copper and organic ligands influences the availability of metals toward β-glucosidase (Geiger et al. 1998). Thus, β-glucosidase activity under integrated nutrient management gets recovered.

Results showed that when the soil of different treatment combinations were exposed to chloroform fumigation, soils of INM plot in particular, INM (½N + ½P + K) of T_3+FYM+BF, T_6 recorded the highest increment in β-glucosidase activity (85.0%) over T_1 followed by organic treatment combination of FYM+BF, T_7 (84.4%); INM (½N + ½P + K) of T_3 +GM+BF, T_5 (56.5%) and INM (½N + P + K) of T_3 +GM, T_4 (25.1%). STCR-based nutrient management, T_3 recorded only 9.8 % increased β-glucosidase activity overT_1 under chloroform fumigation. Thus, result inferred that INM as a whole increase β-glucosidase activity over conventional farming and soil where no fertilizer is applied. But, the overall low response of β-glucosidase under INM systems to chloroform fumigation may be related to selected effect on sensitive microbial population and the growth of resistant species. The latter may feed on cell debris, leading to restructuring of soil microbial population elaborating β-glucosidase enzyme. This finding corroborates the finding of Klose and Ajwa (2004). Furthermore, changes in microbial community under chloroform fumigation may also reduce microbial functionalities through enzyme mediated processes (Nannipiri et al., 2002). For the treatment, T_2, T_5, T_6 and T_7 disturbance duration created by chloroform fumigation lasted upto 63th day of the incubation period and thereafter, β-glucosidase enzyme recovered its activity progressively till the end of the incubation (Fig.3). Where, in case of T_1, T_3 and T_4 inhibition of β-glucosidase activity was restricted upto 84th day. Thus, results revealed that recovery of β-glucosidase activity activity of soil was faster under FYM treated soil in comparison to other treatment options. Speediest rate of recovery (23.7%) was attained in T_6 followed by T_7 and T_2 (18.2% and 9.8%) respectively. The result may be addressed by the fact as discussed earlier in case of other enzyme activity.

3.7 urease activity

Results showed that under no stress condition and when exposed to different stressors, the response of urease activity (µg NH4-N /g soil/hr) of soil under different soil management options was statistically non-significant (Fig.4). However, it shows the similar trend as that of earlier discussed soil enzymes. Soils of organic based farming and FYM/GM-based INM systems maintained highest level of urease enzyme activity than the STCR-based nutrient management and no fertilized plot soils. Less diversified microorganisms under organic resource poor environment failed to restore urease activity under control and STCR-based nutrient management (conventional) in spite of having higher urea-N content in STCR-based nutrient management system.

Soil of STCR-based nutrient management did not attain recovery of urease activity from copper toxicity till the end of the incubation period. Copper has eco-toxicological impacts. Its contamination inhibits the activity of urease (Wyszkowska et al., 2005), showing the sensitive of urease to copper. On the other hand, GM/FYM based nutrient integration was able to recover urease activity of soil at the end of the incubation (Fig.4). This is because of management effect on urease activity under copper stress. FYM-based INM systems as well as green manuring probably shifted the urease expressing microbial communities towards more resistant species. Moreover, soils under INM systems contain more organic colloids adsorbing urease enzyme. The adsorbed urease remains active for larger period even in stressful environment (Winiarski, 1990).

By this mechanism soil organic matter is able to mask the negative impact of Cu on the enzyme activity. Thus, rate of inhibition generally observed by the previous authors did not notice here. Inhibition of urease activity due to chloroform fumigation persists 105th day of incubation. Thus, result inferred that the recovery of urease activity from chloroform fumigation was not attained till the end of the incubation period. This indicates the sensitivity of urease enzyme to chloroform fumigation.

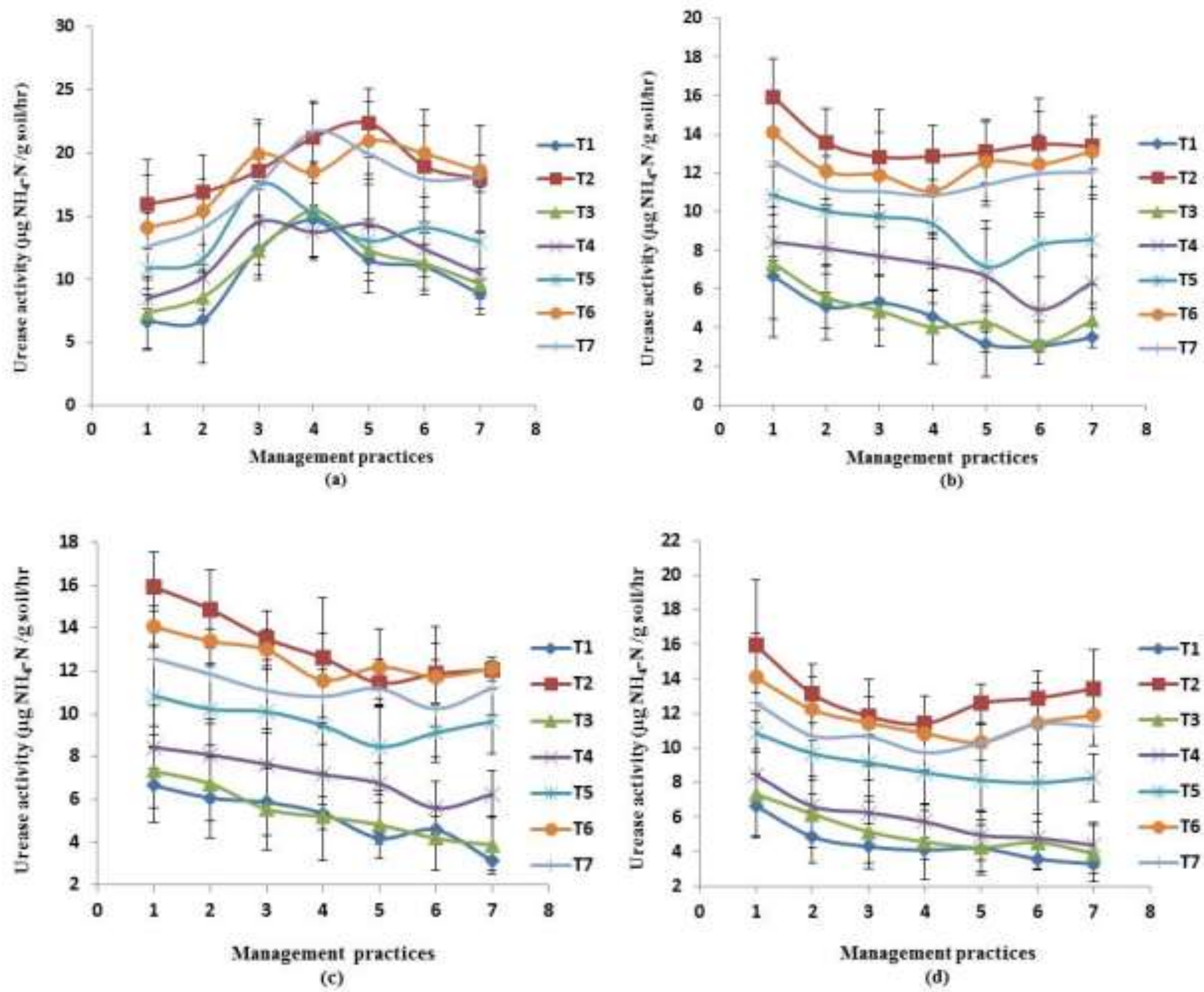

Figure 4. Trend of urease activity (µg NH_4-N /g soil/ hr) of soils under different management practices during 105 days of incubation as influenced by different abiotic stresses *viz*. (a) no stresses (b) heat stress (c) copper toxicity and (d) chloroform fumigation. Error Bars indicate standard deviation of mean.

4. Conclusion

Different biological indicators behaved differently towards different stresses under the influence of diverse management options. In most cases, STCR-based nutrient management consisting of only chemical fertilizers, failed to maintain necessary enzymatic activities and soil functions under different stresses. In contrast, incorporation of chemical fertilizer along with organic sources like Green manure, FYM and biofertilizers maintained relatively higher enzymic activities and soil functions than the sole chemical fertilizer treatment when exposed to different stresses. FYM based nutrient management, particularly, integrated use of ½ N+ ½ P+K of STCR-based fertilizer with FYM (@ 7.5 t /ha) and biofertilizer [*Azospirillum* + Phosphobacter (@4.0 kg/ha)] triggered the highest enzyme activities and soil microbial activities to exert more resistance against the abiotic stressors, than STCR-based fertilizer treatment. After being duly studied in diverse abiotic stress condition for it robustness, the present study infers that INM, comprising of rational use of chemical fertilizers in conjunction with FYM and biofertilizer can be recommended as the best management strategy for better soil health and agricultural sustainability under the upcoming stressed environment.

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