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ORIGINAL PAPER

Impact of elevated CO_2, flooding, and temperature interaction on heterotrophic nitrogen fixation in tropical rice soils

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Abstract Response of N_2 fixation to elevated CO_2 would be modified by changes in temperature and soil moisture because CO_2 and temperature or water availability has generally opposing effects on N_2 fixation. In this study, we assessed the impacts of elevated CO_2 and temperature interactions on nitrogenase activities, readily mineralizable C (RMC), readily available N (NRN) contents in an alluvial and a laterite rice soil of tropical origin. Soil samples were incubated at ambient (370 $\mu mol mol^{-1}$) and elevated (600 $\mu mol mol^{-1}$) CO_2 concentration at 25ºC, 35ºC, and 45ºC under non-flooded and flooded conditions for 60 days. Elevated CO_2 significantly increased nitrogenase activities and readily mineralizable C in both alluvial and laterite soils. All these activities were further stimulated at higher temperatures. Increases in nitrogenase activity as a result of CO_2 enrichment effect over control were 16.2%, 31.2%, and 66.4% and those of NRN content were 2.0%, 1.8%, and 0.5% at 25ºC, 35ºC and 45ºC, respectively. Increases in RMC contents were 7.7%, 10.0%, and 10.6% at 25°C, 35°C and 45°C, respectively. Soil flooding resulted in a more clear impact of CO_2 enrichment than the non-flooded soil. The results suggest that in tropical rice soils, elevated CO_2 increased readily available C content in the soil, which probably stimulates growth of diazotrophic bacteria with enhanced N_2 fixation and thereby higher available N.

Keywords Elevated CO_2 · Heterotrophic nitrogen fixation · Available N · Readily mineralizable carbon · Rice soil

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Introduction

Nitrogen often limits primary production (Cheng et al. 2001). It is believed that submerged rice soil can maintain its fertility for a long time due to soil biological N fixation (BNF) (Ladha et al. 1997), which finds in rice soils two of the most favorable conditions, such as optimum oxygen tension and a constant and regular supply of carbon substrate (Ladha and Reddy 2003). In a future climatic scenario, higher CO_2 concentration coupled with high temperature will affect the ecosystem's carbon–nitrogen balance and SOM decomposition (von Lützow and Kögel-Knabner 2009). However, little is known about the effect of elevated CO_2 concentration on N_2 fixation (Cheng et al. 2001; Hoque et al. 2001; Hungate et al. 2003) and associated diazotrophic communities in tropical paddy soil. Nitrogen fixation is affected not only by substrate but also by soil temperature and moisture (Serraj et al. 1999; Garten et al. 2008). Changes in atmospheric CO_2 concentration, temperature, and water availability can have opposite effects on N_2 fixation (Garten et al. 2008). Studies on the impact of CO_2 concentration, temperature, and moisture level on the extent of BNF in soils are, however, few and far between (French et al. 2009).

The increase in the concentration of tropospheric CO_2 (Kattenberg et al. 1996), coupled with the rise in the other greenhouse gases, is predicted to increase mean global temperature from 1.4ºC to 5.8ºC by the end of the century (IPCC 2007). The impact of elevated CO_2 associated to the rise in ambient temperature with less water availability (Aranjuelo et al. 2007) is supposed to markedly affect biological N_2 fixation. The objectives of the present study were (1) to study the impact of elevated CO_2, temperature, and flooding on biological N_2 fixation, and (2) the influence of nitrogen and carbon availability on BNF under conditions of elevated CO_2, temperature, and flooding in two tropical rice soils.

Materials and methods

Soils

The experiment was carried out with two tropical paddy soils. Soil samples (surface, 0–15 cm) were collected from the rice fields at CRRI, Cuttack (alluvial) and Sukinda (laterite) in the month of May (fallow period between rice–rice cropping system), air-dried in shade, grounded, sieved (>2 mm), and stored in polyethylene bags at room temperature under dry condition. Physicochemical parameters of the soils were determined according to Spark et al. (1996) and were reported in Table 1.

Experimental set-up

Experiments were set-up under two moisture regimes (flooded and non-flooded) by placing 20 g soil in 130-ml pre-sterilized serum bottles and closed with neoprene septa. For the flooded experiment, 20-g soils were flooded with 25 ml sterile distilled water (soil:water:1:1.25) to provide a thin layer (1 cm) of standing water. For the non-flooded experiment, soils were moistened with sterile distilled water to 60% MHC. Elevated CO_2 concentrations in the headspace were set at 600±20 μmol mol^{-1} by replacing the appropriate amount of air in the headspace with the equivalent quantity of 0.2% CO_2 in air. Soil samples in serum bottles were incubated at 25ºC, 35ºC and 45ºC in separate biological oxygen demand (BOD) incubators. Changes in CO_2 concentration in the serum bottles, if any, were monitored at 2-day intervals by sampling 5 ml of headspace gas and quantifying the CO_2 content by absorption in 0.1 N NaOH. The CO_2 concentration was kept constant by injecting the required CO_2 concentration in the headspace of the serum bottles. Changes in CO_2 concentration were never higher than 2%. At 5, 10, 20, 40, and 60 days, nitrogenase activity, and at 10, 20, 40 and 60 days, readily available N and readily mineralizable C content of soil were measured.

Measurements

The acetylene reduction method (Yoshida and Ancajas 1973; Nayak and Rao 1981) was used to measure N fixation activity. Soil sample maintained at different CO_2 concentrations, temperature, and moisture regimes in the butyl rubber stopper-sealed BOD bottle was used. Ten percent (volume) of the headspace air was replaced with pure acetylene gas using a syringe. After the bottles were incubated for 24 h, ethylene production was measured by a gas chromatograph (Varian 3600) with a FID detector (Rao 1976).

Ninhydrin reactive N (NRN) content of soil was measured to determine readily available soil N (Inubushi et al. 1991); it was extracted with 0.5-M K_2SO_4 solution (1:4 *w*/*v*, dry soil/extract ratio) and estimated colorimetrically (Badalucco et al. 1992; Amato and Ladd 1988). Readily mineralizable C content of the soil samples from laboratory incubation was estimated after extraction with 0.5-M K_2SO_4 (Inubushi et al. 1991) followed by wet digestion of the soil extract with dichromate (Vance et al. 1987).

Statistical analysis

Data were subjected to statistical analysis (Gomez and Gomez 1984) by a statistical package (IRRISTAT version 3.1; International Rice Research Institute, Los Baños, Philippines). The mean difference comparison between the treatments was analyzed by analysis of variance (ANOVA) and subsequently by Duncan's multiple range test at $p<0.05$. Simple and multiple correlations between different soil parameters were analyzed by SYSTAT (SPSS Inc. 1999) to establish possible statistical relationship.

Results

Nitrogenase activity increased significantly ($p<0.01$) at elevated CO_2 concentration. Rise in temperature also significantly ($p<0.01$) stimulated nitrogenase activity, whereas BNF activity was higher under flooding than

Table 1 Physicochemical properties of soils

Location	Soil type	pH	EC (dS.m^{-1})	CEC (meq.g^{-1} soil)	Organic carbon (%)	Total Nitrogen (%)	Soil separates		
							Clay (%)	Silt (%)	Sand (%)
Cuttack	Alluvial	6.16	0.50	15.0	0.86	0.09	25.9	21.6	52.5
Sukinda	Laterite	5.90	1.10	6.0	0.76	0.04	14.6	10.6	74.8

under non-flooding conditions (Fig. 1). Increases in nitrogenase activity as a result of CO_2 enrichment effect over control were 16.2%, 31.2%, and 66.4% at 25°C, 35°C, and 45°C, respectively and were statistically significant ($p < 0.05$). The interaction between CO_2 concentration and temperature, and CO_2 concentration, temperature, and flooding was statistically significant (Table 2). Interestingly, nitrogenase activity in submerged alluvial soil was increased up to 40 days of incubation and thereafter decreased gradually. On the contrary, nitrogenase activity of non-flooded alluvial soil and laterite soil was increased up to 20 days of incubation and decreased thereafter (Fig. 1).

Changes in the mean ninhydrin reactive nitrogen (NRN) content in ambient and elevated CO_2 concentration at different temperature and moisture are shown in Fig. 2. The increase in the NRN contents of soil by increasing CO_2 concentration was not significant, whereas it was significant ($p<0.01$) at the elevated temperature at both moisture regimes. The increase in NRN content was higher in flooded than non-flooded soil. The percentage increase in the NRN content due to the elevated CO_2 effect over control was 2.0%, 1.8%, and 0.5% at 25°C, 35°C, and 45°C, respectively and the relative effect was not significantly different at 25°C and 35°C. Though the interaction between temperature and moisture was significant, the interaction between CO_2 concentration and temperature, CO_2 concentration and moisture and among CO_2 concentration, temperature and moisture were not statistically significant (Table 2). As already stated, the NRN content was lower in non-flooded than flooded soil, and it decreased gradually with incubation in both moisture regimes in the two soils (Fig. 2).

The mean readily mineralizable C (RMC) at ambient and elevated CO_2 concentration at different temperatures and moistures are shown in Fig. 3. It increased significantly ($p<0.01$) at elevated CO_2 concentration and by increasing temperature ($p<0.01$), and it was higher in the flooded than in non-flooded soil. Increases in RMC content as a result of CO_2 enrichment effect over control were 7.7%, 10.0%, and 10.6% at 25°C, 35°C, and 45°C, respectively, and the relative effect was not significantly different at 35°C and 45°C. The increase in RMC content was statistically significant over days, temperature, CO_2 concentration, and moisture regimes independently. Though the interaction between CO_2 concentration and temperature was not significant, interaction between CO_2 concentration and moisture, temperature and moisture, and among CO_2

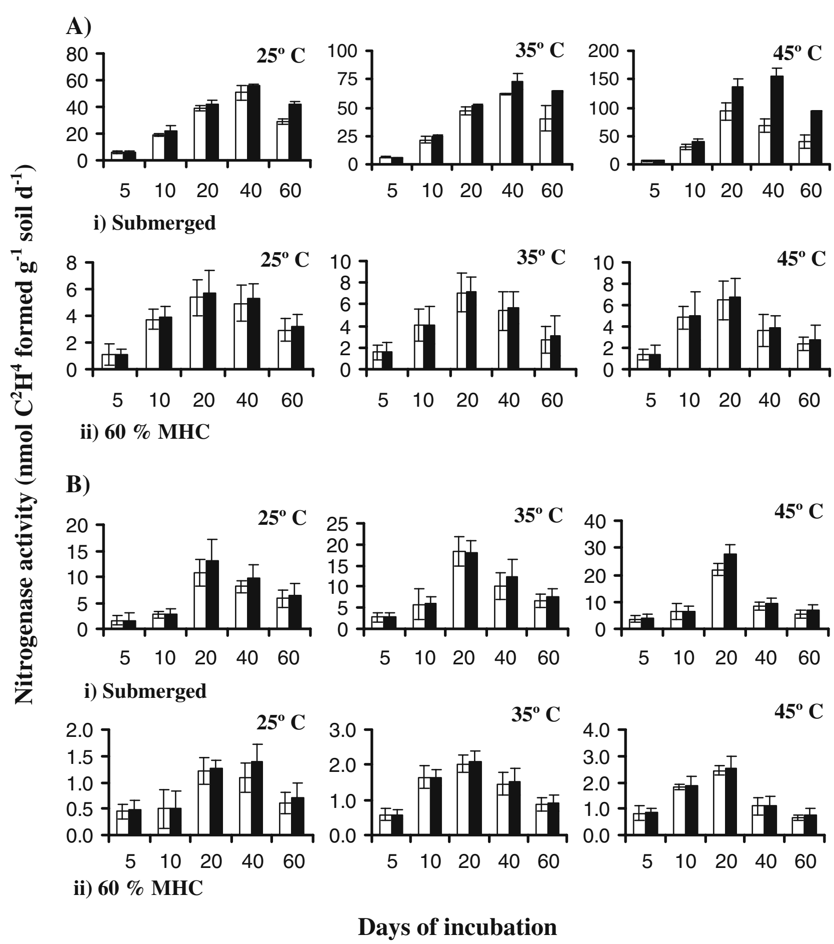

Fig. 1 Nitrogenase activity of **A** alluvial soil and **B** laterite soil at two moisture regimes (*i*) submerged and (*ii*) 60% MHC and three temperatures (25°C, 35°C, and 45°C). Mean of three replicate values plotted, *bars*/*half bars* indicate the standard deviation (*white bars* control, *black bars* 600 μmol.mol^{-1})

Table 2 *F* value of soil and microbial parameters as influenced by the analysis of variance

Sources of variation	Soil parameters		
	ARA	NRN	RMC
C	167.80**	3.77 ns	112.23**
T	212.86**	714.60**	162.90**
W	3,884**	15,680**	2,364**
C×T	41.63**	0.27 ns	2.12 ns
C×W	159.58**	0.25 ns	4.13*
T×W	201.35**	79.87**	12.45**
C×T×W	41.79**	0.04 ns	7.20**

ANOVA factors were CO_2 concentration, temperature, moisture, days of incubation, and soil type

C CO_2 concentration, *T* temperature, *W* water regime, *ARA* acetylene reduction assay (nitrogenase activity), *NRN* ninhydrin reactive nitrogen, *RMC* readily mineralizable carbon, *ns* not significant

*$p<0.05$, **$p<0.01$

concentration, temperature and moisture was statistically significant ($p<0.01$; Table 2). Readily mineralizable C content of both alluvial and laterite soils increased with incubation, reaching the highest level at 20 days of incubation and then decreased gradually (Fig. 3). Compared to the non-flooded conditions, RMC contents of soils incubated under flooded condition were influenced by the interactive effect of CO_2 concentration and temperature.

Discussion

The finding of this study can be summarized in the following ways: (1) elevated CO_2 significantly increased nitrogenase activity, and the increase was significant with the rise in temperature. The interaction of elevated CO_2 and temperature had a significant impact on nitrogenase activity. (2) Readily available N in soil was not affected by elevated CO_2 concentration as compared to ambient CO_2 concentration. On the contrary, elevated temperature significantly increased readily available N content of soil, although interaction of elevated CO_2 and high temperature did not show any significant impact on readily available N

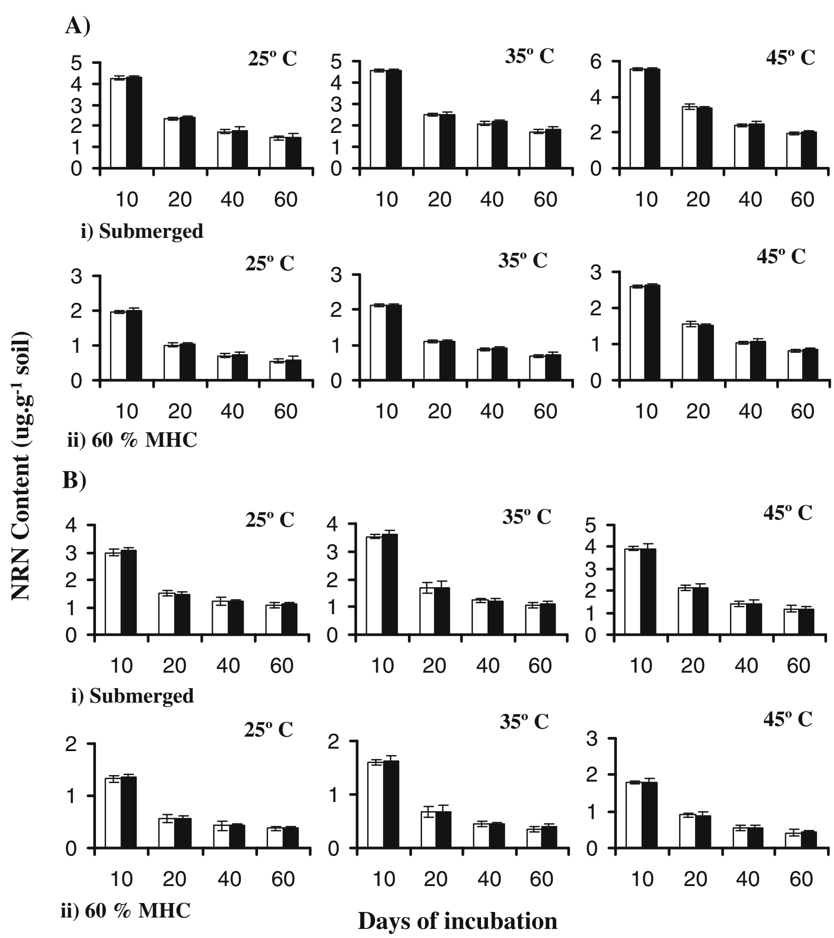

Fig. 2 Ninhydrin nitrogen content of **A** alluvial soil and **B** laterite soil at two moisture regimes (*i*) submerged and (*ii*) 60% MHC and three temperatures (25°C, 35°C, and 45°C). Mean of three replicate values plotted, *bars*/*half bars* indicate the standard deviation. (*white bars* control, *black bars* 600 μmol.mol^{-1})

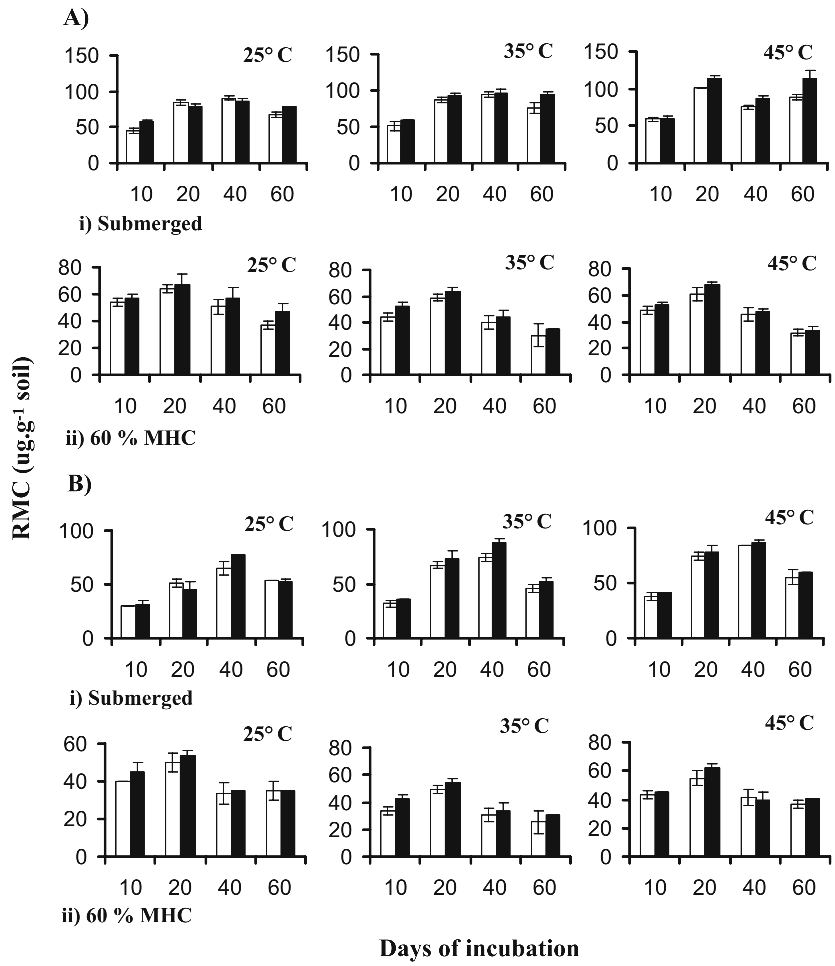

Fig. 3 Readily mineralizable carbon content of **A** alluvial soil and **B** laterite soil at two moisture regimes (*i*) submerged and (*ii*) 60% MHC and three temperatures (25°C, 35°C, and 45°C). Mean of three replicate values plotted, *bars*/*half bars* indicate the standard deviation (*white bars* control, *black bars* 600 μmol.mol^{-1})

content of soil. (3) Elevated CO_2 significantly increased RMC contents of soil, and increase was significant over rise in temperature, albeit the interaction of elevated CO_2 and temperature on RMC content of soil being not significant. (4) The flooded soil responded more positively to the CO_2 enrichment than the non-flooded soil.

As carbon and nitrogen cycles are tightly coupled, it is evident that continued C sequestration would require additional input of N (Hungate et al. 2003). Gifford (1994) hypothesized that this can be provided by BNF and atmospheric deposition. Generally, BNF is enhanced by organic matter application to rice soil due to the supply of available C to soil microorganisms (Rao 1976; Charyulu et al. 1981; Adachi et al. 1989). In our experiment, a significant increase in BNF in response to elevated CO_2 could be due to increase in C input to soil. Indeed, the increase in BNF was positively correlated ($p<0.01$) with the increase in RMC content, which is an indicator of readily available C for microbial metabolism. Ninhydrin reactive nitrogen (NRN), an index of labile N in the rhizosphere soil (Joergensen and Brooks 1990), was positively and significantly correlated with nitrogenase activity ($p<0.01$), probably because the increased labile C inputs into the soil in response to elevated CO_2 stimulated microbial activity and increased soil N availability (Zak et al. 1993; Martin-Olmedo et al. 2002).

The rise in temperature has been shown to reduce N_2 fixation due to lower soil moisture availability (Serraj et al. 1999). We have found that a rise in temperature significantly ($p<0.01$) increased nitrogenase activity which reached the highest value at 45°C in the flooded soil. This can be attributed to maximum RMC content at 45°C in flooded soil. It is interesting to note that though the nitrogenase activity reached a peak value at 45°C in flooded soil, in non-flooded soil, it reached a peak value at 35°C. This can be due to the metabolic responses of different diazotrophic bacterial population to temperature and readily available carbon. The tropical soil used in this study could have had microbial population acclimatized to high temperature, and these populations can be further stimulated under specific moisture regimes and elevated CO_2 concentration.

Nitrogen fixation appears to be sensitive to water stress. The low N_2 fixation under non-flooded condition could be attributed to high oxygen tension, which is known to inhibit nitrogenase activity (Brouzes et al. 1971). On the other hand, submerged soils provide adequate moisture, nutrient supply, and favorable aeration conditions for the activity of N_2 fixers (Rao 1976). In our experiment, we observed that flooding the soil responded more effectively to the CO_2 enrichment than the non-flooded soil. The interaction between water regime and CO_2 significantly influenced nitrogenase activity ($p<0.01$) and RMC contents ($p<0.05$), whereas it did not have significant impact on NRN contents, indicating flooding at elevated CO_2 enhanced readily available C than the readily available N.

In the present study, we have observed increase in nitrogenase activity in tropical rice soils in response to elevated CO_2 and temperature interactions. It was observed that elevated CO_2 increased readily available C input into the soil, which in turn stimulated diazotrophic bacteria to proliferate and hence enhanced N_2 fixation in tropical rice soil, especially under flooded conditions. However, this finding should be interpreted with caution because these responses were observed during a short-term incubation study. Long-term studies are needed to expand the horizon of our knowledge on the responses of diazotrophic communities and N_2 fixation to elevated CO_2 and temperature interaction.

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References

Adachi K, Watanabe I, Kobayashi M, Takahashi E (1989) Effect of application of glucose, cellulose and rice straw on nitrogen fixation (acetylene reduction and soil nitrogen components in anaerobic soil. Soil Sci Plant Nutr 35:235–243

Amato M, Ladd JN (1988) Assay of microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biol Biochem 20:107–114

Aranjuelo I, Irigoyen JJ, Diaz MS (2007) Effect of elevated temperature and water availability on CO_2 exchange and nitrogen fixation of nodulated alfalfa plants. Environ Exp Bot 59:99–108

Badalucco L, Grego S, Dell’Orco S, Nannipieri P (1992) Effect of liming on some chemical, biochemical, and microbiological properties of acid soils under spruce (*Picea abies* L.). Biol Fertil Soils 14:76–83

Brouzes R, Mayfield CI, Knowles R (1971) Effect of oxygen partial pressure on nitrogen fixation and acetylene reduction in a sandy loam soil amended with glucose. Plant Soil 35:481–494

Charyulu PBBN, Nayak DN, Rao VR (1981) $^{15}N_2$ incorporation by rhizosphere soil influence of rice variety, organic matter and combined nitrogen. Plant Soil 59:399–405

Cheng W, Inubushi K, Yagi K, Sakai H, Kobyashi K (2001) Effects of elevated carbon dioxide concentration on biological nitrogen fixation, nitrogen mineralization and carbon decomposition in submerged rice soil. Biol Fertil Soils 34:7–13

French S, Levy-Booth D, Samarajeewa A, Shannon KE, Smith J, Trevors JT (2009) Elevated temperatures and carbon dioxide concentrations: effects on selected microbial activities in temperate agricultural soils. World J Microbiol Biotechnol 25:1887–1900

Garten CT Jr, Classen AT, Norby RJ, Brice DJ, Weltzin JF, Souza L (2008) Role of N_2-fixation in constructed old-field communities under different regimes of [CO_2], temperature, and water availability. Ecosystems 11:125–137

Gifford RM (1994) The global carbon-cycle—a viewpoint on the missing sink. Aust J Plant Physiol 21:1–15

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. John Wiley and Sons.Inc, Singapore

Hoque MM, Inubushi K, Miura S, Kobayashi K, Kim HY, Okada H, Yabashi S (2001) Biological dinitrogen fixation and soil microbial biomass carbon as influenced by free-air carbon dioxide enrichment (FACE) at three levels of nitrogen fertilization in a paddy field. Biol Fertil Soils 34:453–459

Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB (2003) Nitrogen and climate change. Science 302:1512–1513

Inubushi K, Brookes PC, Jenkinson DS (1991) Soil microbial biomass C, N and ninhydrin-N in aerobic and anaerobic soils measured by fumigation-extraction method. Soil Biol Biochem 23:737–741

IPCC (2007) Summary for policymakers. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, HL Miller M (eds) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York, pp 1–18

Joergensen RG, Brooks PC (1990) Ninhydrin reactive nitrogen measurements of microbial biomass based on ninhydrin reactive nitrogen in extracts of fumigated soil. Soil Biol Biochem 22:1023–1027

Kattenberg A, Giorgi F, Grassl H, Meehl GA, Mitchell JFB, Stouffer RJ, Tokioka T, Weaver AJ, Wigley TML (1996) Climate models—projections of future climate. In: Houghton JT, and others (eds) Climate change 1995: the science of climate change. Report of IPCC Working Group I. Cambridge University Press, Cambridge, pp 289–357

Ladha JK, Reddy PM (2003) Nitrogen fixation in rice system: state of knowledge and future prospects. Plant Soil 252:151–167

Ladha JK, de Bruijn FJ, Malik KA (1997) Introduction: assessing opportunities for nitrogen fixation in rice—a frontier project. Plant Soil 194:1–10

Martin-Olmedo P, Rees RM, Grace J (2002) The influence of plants grown under elevated CO_2 and N fertilization on soil nitrogen dynamics. Glob Chang Biol 8:643–657

Nayak DN, Rao VR (1981) Influence of alternate flooded and non-flooded conditions on nitrogen fixation (C_2H_2 reduction) in paddy soils. Soil Sci 131:26–29

Rao VR (1976) Nitrogen fixation as influenced by moisture content, ammonium sulphate and organic C sources in a paddy soil. Soil Biol Biochem 8:445–448

Serraj R, Sinclair TR, Purcell LC (1999) Symbiotic N2 fixation response to drought. J Exp Bot 50:143–155

Spark DL, Page AL, Summer ME, Tabatabai MA, Helmke PA (1996) Methods of soil analysis, Part 3, Chemical methods. American Society of Agronomy, USA, p 1358

SPSS Inc (1999) SPSS for windows, Release 10. Chicago IL

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass. Soil Biol Biochem 19:703–707

von Lützow M, Kögel-Knabner I (2009) Temperature sensitivity of soil organic matter decomposition—what do we know? Biol Fertil Soils 46:1–16

Yoshida T, Ancajas R (1973) Nitrogen fixation activity in upland and food rice field. Soil Sci Soc Am Proc 37:42–46

Zak DR, Pregizer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. Plant Soil 151:105–117