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Impact of phosphorus supply on root exudation, aerenchyma formation and methane emission of rice plants

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Abstract. This study evaluated the impact of P supply on rice plant development and the methane budget of rice fields by 2 different approaches: (1) root growth, exudation and aerenchyma formation were recorded in an experiment with hydroponic solution; (2) dissolved CH_4 concentration and CH_4 emission were investigated in a pot experiment. In both approaches, we used three different cultivars and three levels of P supply. In the experiment with solution culture (0.5 ppm, 5 ppm, and 10 ppm P), root exudation ranged between 0.5 to 36.7 μmol C plant^{-1} h^{-1} and increased steadily with plant growth at given P level. Low P supply resulted in

- depressed shoot growth but increased root growth in culture solution,
- increments in the root/shoot ratio by factors of 1.4 to 1.9 at flowering stage,
- enhanced the development of root aerenchyma, and
- stimulation of root exudation per plant by factors of 1.3–1.8 as compared to medium P supply and by factors of 2.1–2.4 as compared to high P supply.

However, root exudation did not differ among treatments when related to the dry weight of roots. Thus, high exudation rates were caused by larger root biomass and not by higher activity of the root tissue.

The pot experiment was conducted with a P-deficient soil that was either left without amendment or fertilized by 25 and 50 mg P kg_{soil}^{-1}, respectively. Low P supply resulted in

- higher CH_4 concentrations in soil solution; i.e., at flowering stage the soil solution concentrations were 34–50 μM under P deficiency and 10–22 μM under ample P supply and
- significant increases of CH_4 emission rates during the later stages of plant growth.

These findings reflect a chain of response mechanisms to P stress, that ultimately lead to higher methane emission rates.

Introduction

The increase of methane in the atmosphere contributes to global warming and affects the photo-chemistry of the atmosphere (Cicerone & Oremland 1988). Wetland rice soils have been shown to be an important source of atmospheric methane at the global scale (Bartlett & Harriss 1993; IPCC 1995). Rice plants play a major role in the CH_4 flux from ricefields (Neue & Sass 1994; Schütz et al. 1989; Wassmann & Aulakh 1998).

Several studies have shown that up to 90% of the methane released from the rice fields to the atmosphere is emitted through the rice plants (Seiler et al. 1984; Holzapfel-Pschorn et al. 1986; Butterbach-Bahl et al. 1997). The well-developed aerenchyma in rice plants provides an effective channel for gas exchange between atmosphere and the anaerobic soil (Nouchi 1990; Butterbach-Bahl et al. 1997). While the aerenchyma serves as conduit for CH_4 from the anoxic soil to the atmosphere, it transports O_2 in the reverse direction as well (Armstrong 1979). Methane oxidation in rhizosphere accounts for up to 95% of the potential CH_4 emission (Schipper & Reddy 1996; Gilbert & Frenzel 1995; Epp & Chanton 1993; King 1994). Kludze et al. (1993) evaluated aerenchyma formation in response to soil reduction status. However, fewer studies considered the effect of nutrient supply (Kirk & Du 1997).

In addition to their function as a conduit for gas exchange, rice plants also act as major suppliers of substrates for CH_4 formation in rice fields during the growing season. On average, 30–60% of the net photosynthetic carbon is allocated to the roots and, substantial proportion of this carbon is released in form of organic compounds into the rhizosphere (Marschner 1996). Several studies speculated that the maximum peaks and/or increases of methane emission rates at later stages of plant growth might be caused by increase of root exudates or root autolysis products (Holzapfel-Pschorn et al. 1986; Lindau et al. 1991; Neue & Sass 1994; Chidthaisong & Watanabe 1997). The quantitative evidence for the role of the plant-borne carbon has also been provided recently by $^{13}CO_2$ labeling approach (Kimura 1997; Minoda & Kimura 1994). Rhizodeposition of organic carbon increased by various stresses including nutrient deficiency (Lynch 1990). Enhanced root exudation of organic carbon, organic acids in particular, was often observed under phosphorus deficiency in various kinds of crops (Marschner 1996). Up to 2–5 times more rhizodeposition of organic acids have been reported under phosphorus stress (Lipton et al. 1987; Hoffland et al. 1989). However, a recent review by Wassmann and Aulakh (1998) revealed limited information on relationship between CH_4 emission and root exudation as regulated by plant nutrient status. In this study, we evaluated the effects of phosphorus supplies on root development, root exudation, aerenchyma formation and CH_4 production and emission.

Materials and methods

Experiment I: Hydroponics experiment

Plant culture

Experiments were conducted with three rice cultivars varying in susceptibility to P deficiency; i.e. IR26 was identified as tolerant, IR72 as moderately susceptible and IR36 as highly susceptible to P deficiency. Sterilized and healthy germinated seeds were sown in 4-liter pots containing culture solution with different P levels. The black pots were covered by black PVC lids with 4 holes (20 mm i.d.) allowing the growth of rice plants on nylon nets. All plants were grown in the greenhouse with the following conditions: day temperatures ranged from 32 °C to 34 °C, while night temperatures were between 28 °C and 30 °C; day-time solar radiation varied from 1300 μmol m^{-2} s^{-1} to 1450 μmol m^{-2} s^{-1}; relative humidity ranged between 80% and 85% throughout the growth period. The culture solution was replenished daily by de-ionized water, adjusted at pH 5.0 using 1 N NaOH or 1 N HCl every 2 days and renewed twice a week. The nutrient solution (Yoshida et al. 1976) consisted of: 40 mg L^{-1} N as NH_4NO_3, 40 mg L^{-1} K as K_2SO_4, 40 mg L^{-1} Ca as $CaCl_2$, 40 mg L^{-1} Mg as $MgSO_4$, and traces of Mn, Mo, B, Zn, Cu, and Fe (in the form of Fe-EDTA). In addition one treatment received 10 mg L^{-1} P (as $NaH_2PO_4.H_2O$) the other 2 treatments received 5 and 0.5 mg L^{-1} P, respectively. Experiments were carried out with three replications in completely randomized block design.

Collection of root exudates

Root exudates were collected at 14, 28, 47, 64 and 76 days after sowing. At each sampling date, roots were rinsed with de-ionized water. The plants were transferred to 500 ml Erlenmeyer flask with the root hanging into 500 ml sterilized de-ionized water. Flasks were completely covered with aluminum foil and were transferred to a growth chamber with the following conditions: temperature 29 °C; photosynthetic photon flux density 1200 mmol m^{-2} s^{-1} at the top of the plants, and a relative humidity (RH) 80%. After 5 h exposure, the solutions were collected and successively filtered through #42 filter and 0.45 μm membrane filters to remove dead cells, debris and microorganisms. The filtrates were immediately frozen and subsequently concentrated 20-fold by freeze-drying in a lyophilizer. All samples were kept frozen until analysis.

Measurement of carbon in root exudate

Soluble organic carbon of root exudates was determined by a protocol according to Page et al. (1982) with a slight modification. In brief: 5 ml of root exudates, 5 ml of 0.035 N $K_2Cr_2O_7$, 10 ml of 98% H_2SO_4 and 5 ml of 88%

H_3PO_4 were kept in a digestion tube at 150 °C for 30 minutes. Then, the solution was cooled, transferred to a 150 ml Erlenmeyer flask, and titrated with 0.005 N $Fe(NH_4)_2(SO_4)_2 \times 6\ H_2O$ in 0.4 M H_2SO_4 solution. Sucrose was used as reference substance.

Measurements of root porosity

After collecting the root exudates, fresh roots were used for measurements of root porosity by the pycnometer method (Jensen et al. 1969; Kludze et al. 1993). Fresh roots (1.0 g) were gently blotted dry on tissue paper and to determine the fresh weight (W_r) on an analytical balance. The sample was then placed in a pycnometer bottle. The bottle was filled with water, taped and weighed (W_{r+w}). The roots were later retrieved, ground with mortar and pestle, and the resulting homogenate of the roots was returned to the bottle. The bottle was then toped up with water and weighed again (W_h). The bottle was also weighed filled with water only (W_w). The relative porosity (RP) of the roots was then calculated with the formula:

$$RP\ (\%) = 100\ (W_h - W_{r+w})/(W_w + W_r - W_{r+w}). \quad (1)$$

Root and shoot biomass were also recorded after oven drying.

Experiment II: Soil pot experiment

Soil, plant and fertilizer material

The soil used was a phosphorus-deficient soil (Inceptisol) collected from the plow layer of a paddy field in Pangil, Laguna Province, Philippines. Soil was air dried, ground, and then thoroughly mixed. Root debris was carefully removed. The air-dried soil had pH 4.9 (1:1, soil/water ratio), CEC 5.0 $cmol_c\ kg^{-1}$, available P 2.2 mg kg^{-1}, and total C and N contents 3.43% and 0.225%, respectively. Experiments were conducted using same three cultivars (IR26, IR36 and IR72) as in solution culture experiment. Sterilized seeds were first grown on culture solution (25% of the concentrations listed above) for 2 weeks. Three seedlings were then transplanted to a 4-liter pot that contained 3.0 kg Pangil soil. The soil was thoroughly mixed with 25 mg N/kg soil (as urea) and 12.5 mg K/kg soil (as muriate potash) before transplanting. P was supplied in form of solophos. The treatments of P levels were 0, 25 and 50 mg P/kg soil. After 1 week, plants were reduced to single seedling per pot.

Methane emission measurements

The measurements of methane emission and production started 3 weeks after transplanting. Methane emissions were measured using the closed chamber technique in greenhouse with the same environmental conditions as in the

solution culture experiment. The gas collection chamber made of transparent plastic was 100 cm high and had a diameter of 20 cm. The chamber was equipped with a small fan for air mixing and a rubber septum fixed in its wall for gas sampling by syringes. At each sampling time, the experimental pots were placed in a bigger pot filled with water. The water in the bigger pot was used to seal the bottom of a gas collection chamber that was temporarily placed over individual plant.

Sampling was consistently conducted between 10:00 to 11:00 a.m. Five ml of gas sample was taken at 0, 5, 15, 20 min duration of plant enclosure. Samples were analyzed in gas chromatograph equipped with a flame ionization detector (at 150 °C) using Porapak N column (at 60 °C) and N_2 as carrier gas.

Rates of methane emission were determined from the linear regression of the temporal increase in chamber methane concentration. Data were discarded if r^2-values were less than 0.90 (n = 4). Emission rates were expressed with unit of mg CH_4 d^{-1} $plant^{-1}$, although the total emission was from both plant and pot soil. Gas volume was adjusted with temperature changes inside the chamber, whereas the effect of increasing chamber temperature on flux rate was neglected.

Dissolved methane concentration in soil solution

The technique for collecting soil solution described in Alberto et al. (1997) was modified for pot experiments. In each pot, a porous ceramic tube (Rhizon soil moisture sampler) was inserted vertically in the soil (1–15 cm depth) where it remained throughout the growth season. After each gas sampling, a vacutainer tube was connected to the sampler through a needle and collected 5ml of soil solution. Then, the vacutainer was detached from the sampler. One ml of gas sample from the headspace of vacutainer was analyzed for methane concentration. The concentration of dissolved CH_4 was calculated with the formula:

$$C_s\ (\mu g/ml) = \{C_h[V_h + PV_s] - C_aV_h\}D/V_s, \quad (2)$$

where C_s is concentration of CH_4 (μg/ml) in soil solution, C_h is the concentration of CH_4 (ppm_v) in headspace, C_a is the concentration of CH_4 (ppm_v) in ambient air, V_h is the volume (ml) of headspace, V_s is the volume (ml) of soil solution, P is the partition coefficient (= 0.03 ml air/ml water at lab temperature); D is the density of methane (= 0.00065 g/ml at lab temp.).

Table 1. Root/shoot ratio of three cultivars in culture solutions with low (LP_C = 0.5 ppm P), medium (MP_C = 5 ppm P), and high (HP_C = 10 ppm P) phosphorus supply at different plant ages.

Plant age (d)		14	28	47	76
IR26	LP_C	0.20	0.23	0.18	0.10
	MP_C	0.22	0.17	0.11	0.07
	HP_C	0.21	0.13	0.12	0.07
IR36	LP_C	0.29	0.27	0.18	0.12
	MP_C	0.26	0.22	0.14	0.06
	HP_C	0.27	0.14	0.14	0.06
IR72	LP_C	0.21	0.22	0.15	0.10
	MP_C	0.19	0.13	0.17	0.07
	HP_C	0.21	0.10	0.11	0.05

Results

Experiment I: Hydroponics experiment

Plant growth

Low P supply affected plant growth in culture solution, but the impact differed between root and shoot growth (Figure 1). A decrease in shoot growth under P deficiency was discernable starting at a plant age of 28 days. However, root growth showed the inverse relationship, i.e., plants grown under P deficiency developed more root biomass. For shoot as well as root development the disparity among treatments is clearly illustrated by comparing root/shoot ratios of 14-day plants with those of older plants (Table 1).

Root exudation

The rates of root exudation ranged between 0.5 to 36.7 μmol C $plant^{-1}$ h^{-1} and increased steadily with plant growth at given P level (Figure 2(a, b, c)). Maximum exudation rates were recorded at flowering stage, which were 13–36 times higher than those at early tillering stage. Low P supply enhanced root exudation (Figure 2(a, b, c)). This effect became more prominent with plant growth. Low P supply stimulated root exudation by factors of 1.3–1.8 as compared to medium P supply and by factors of 2.1–2.4 as compared to high P supply. Among cultivars root exudates increased in the order of IR26 > IR36 > IR72.

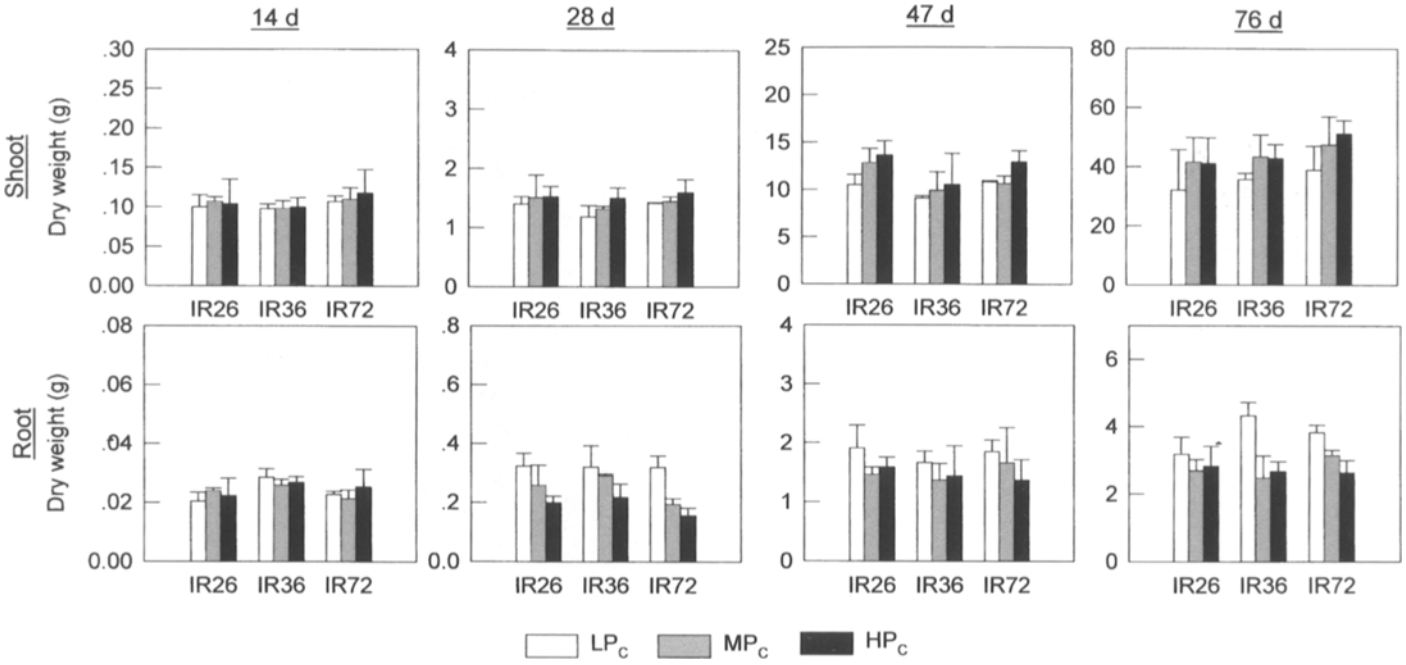

Figure 1. Dry weights of root and shoot of three cultivars under 3 levels of P treatments: 0.5 ppm P (LP_C), 5 ppm P (MP_C and 10 ppm P (HP_C) in solution culture. Bars represent standard deviation.

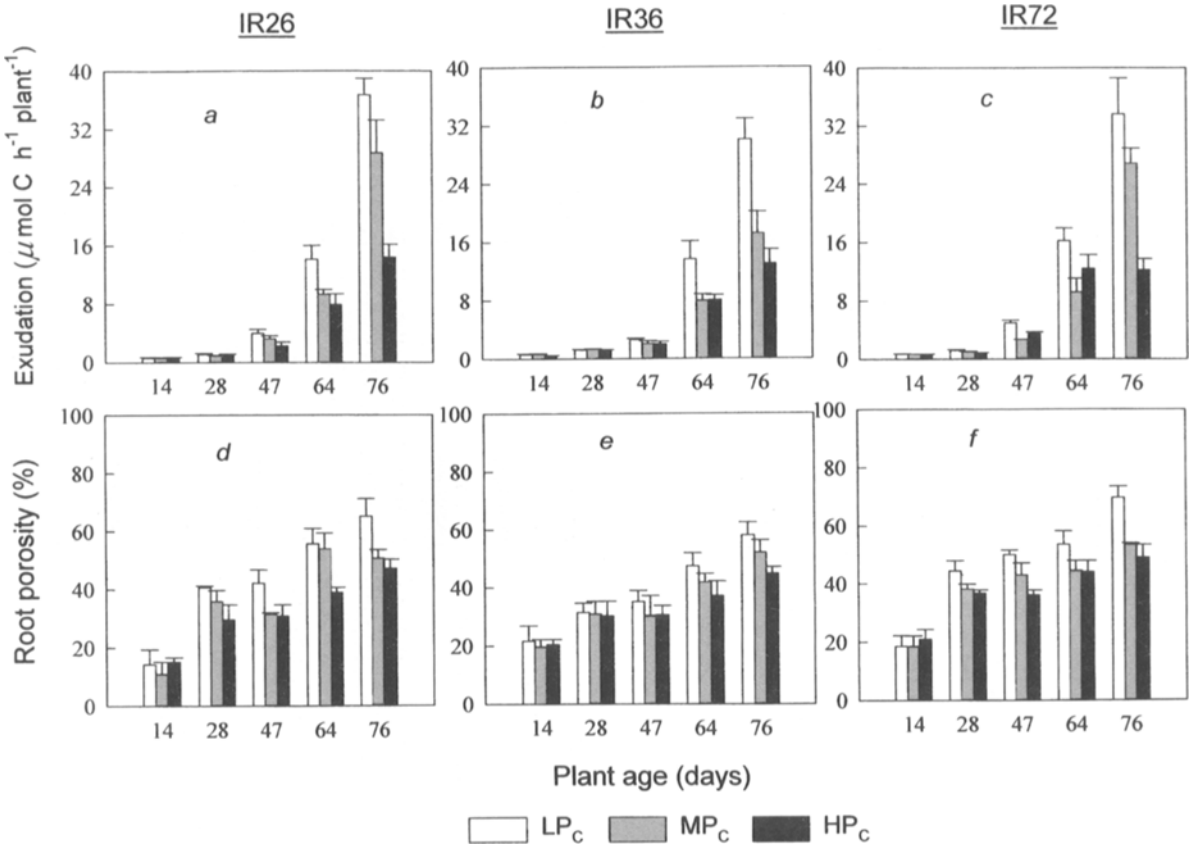

Figure 2. Root exudation rates and relative root porosity of three cultivars under three levels of P treatments: 0.5 ppm P (LP_C), 5 ppm P (MP_C and 10 ppm P (HP_C) in solution culture. Bars represent standard deviation.

Table 2. Mean dissolved CH_4 concentration and CH_4 emission rates of three cultivars on a P-deficient soil treated with 25 mg P kg^{-1} soil (MP_S), 50 mg P kg^{-1} soil (HP_S). Control left without P fertilization (LP_S).

P levels	LP_S	MP_S	HP_S
	Mean dissolved CH_4 (μM)*		
IR26	57 ± 6.9	30 ± 5.4	29 ± 2.1
IR36	52 ± 7.7	29 ± 6.2	35 ± 7.2
IR72	40 ± 5.4	24 ± 4.8	22 ± 5.1
	Mean emission rates (mg CH_4 d^{-1} $plant^{-1}$)		
IR26	3.2 ± 0.34	2.7 ± 0.34	2.4 ± 0.39
IR36	3.5 ± 0.50	2.7 ± 0.30	2.8 ± 0.21
IR72	3.8 ± 0.42	3.3 ± 0.47	3.2 ± 0.37

Note: * Values are mean + standard deviation.

Root porosity

Figure 2(d–f) depicts root air-space formation as a function of plant age and P supply. Root porosity increased with plant growth and ranged between 11–69% of fresh root volume. Similar to root exudation, root porosity also increased in response to P stress.

Experiment II: Soil pot experiment

Dissolved methane in soil solution and methane emission rates were determined starting from a plant age of 35 days which corresponded to 21 days after transplanting. The seasonal patterns were similar in all treatments, i.e., flux rates remained relatively high during tillering (40–70 days) and heading stage (70–85 days) and decreased in ripening stage (after 85 days) (Figure 3(d–f)). However, even at a young plant age of 35 days, dissolved methane already showed significant differences among treatments (Figure 3(a–c)). The pots without P treatment had consistently higher methane concentrations in soil solutions than the P-amended pots. The mean value of dissolved methane concentration in pots without P treatment was significantly higher than in pots amended with P ($P < 0.01$), while no significant difference was found between the two levels of P fertilization, i.e., 25 mg P and 50 mg P per kg soil (Table 2).

The seasonal variations in CH_4 emission encompassed alternating increase/decrease periods (Figure 3(d–f)). The peaks at plant ages of 59 days and 78 days correspond to maximum tillering and flowering stage,

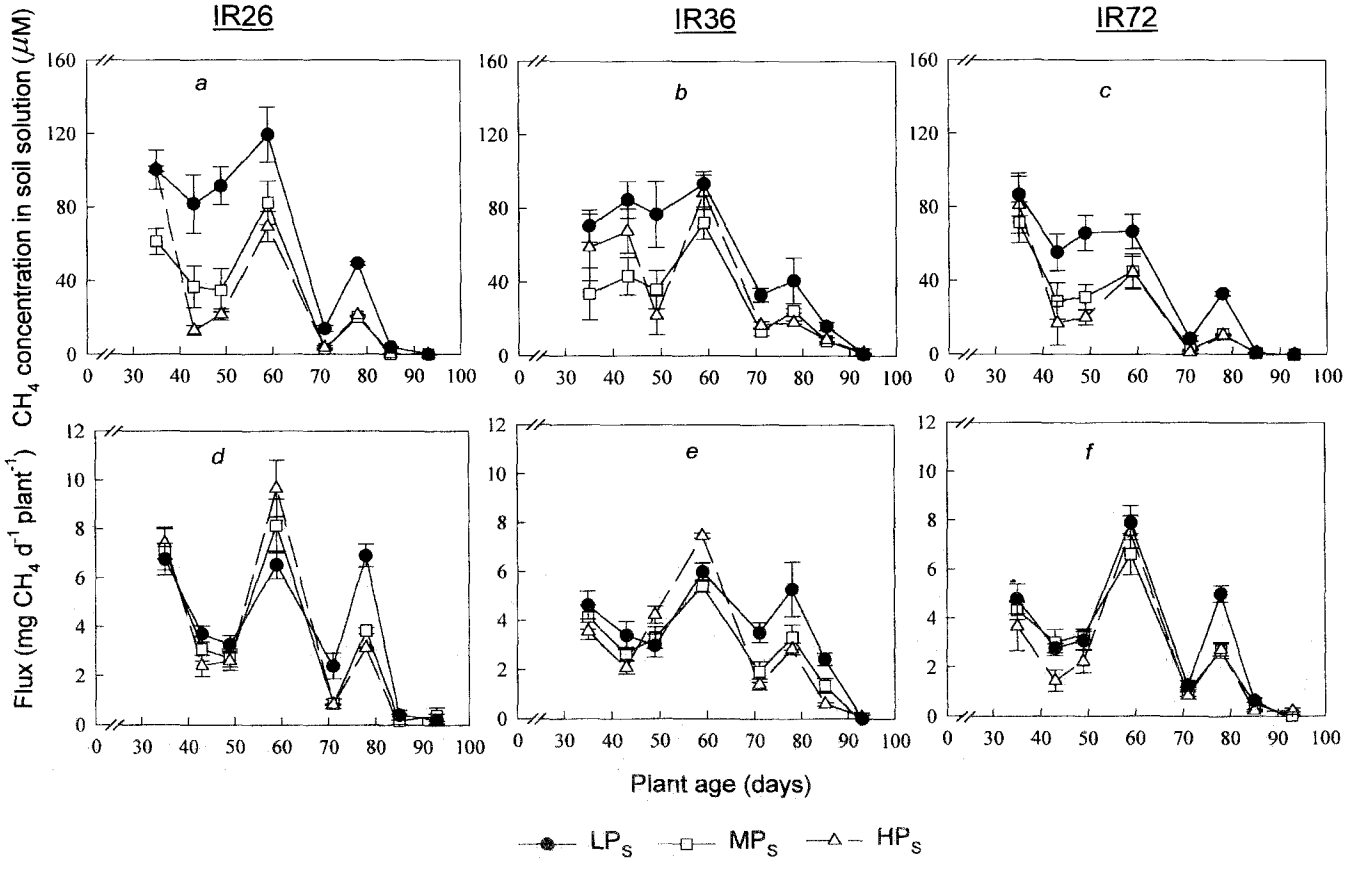

Figure 3. Meethane emission rates and dissolved CH_4 concentration in Pangil soil with three cultivars, treated with 25 mg P kg^{-1} soil (MP_S), 50 mg P kg^{-1} soil (HP_S) and control (LP_S) left without P fertilization. Bars are standard deviation.

respectively. In contrast to dissolved methane, emission rates did not show any treatment effect during early stages. However, during later stages, P deficiency resulted in higher emission rates (Figure 3(d–f)) which is also reflected in the mean values (Table 2). For the three cultivars, the mean emission rates in without-P treatment were 19–33% higher than in those with P fertilization (Table 2) ($P < 0.01$).

Figure 4 displays the status of various parameters at the flowering stage of the rice plant. These values correspond to the last available records for culture solution experiment whereas the soil pot experiment was continued for two more sampling dates. The differences among treatments in plant growth, root exudation, and root porosity were fully developed at this stage: root/shoot ratio differed by 41–71% ($P < 0.001$), root porosity by 26–35% ($P < 0.01$), and exudation rates in low P treatment were 2.2–2.8 fold higher than in high P treatments. The plant stage presented in Figure 4 also corresponds to the last peak in methane emission and dissolve methane. The values of methane emission peaks in without-P treatment were 1.8–2.2 fold higher and the dissolved methane concentrations were 2.2–3.1 fold higher than those with P fertilization, respectively (Table 2).

Discussions

In hydroponics experiments, low P supply depressed shoot growth while root growth was stimulated. Subsequently, the root/shoot ratio was significantly increased in the low P treatment. Root/shoot ratio is an index of the partitioning of photosynthesized carbon between aboveground and belowground sections of the plant. P deficiency generally leads to higher root/shoot ratio (Lassens & Folscher 1988; Marschner 1996; Kirk & Du 1997), whereas it may not always be associated by higher root biomass as found in this experiment with hydroponic solution. In low P soil, both shoot and root growth can substantially be suppressed (Lassens & Folscher 1988).

Numerous studies have shown that P stress caused higher root exudation (Lipton et al. 1987; Hoffland et al. 1989; Marschner 1996). In this experiment, the root exudations of plants were increased by a factor of 1.3–2.4 when grown under low P supply. The stimulation of root exudation under P starvation could be due to lower membrane permeability (Ratnayake et al. 1978; Graham et al. 1981; Lipton et al. 1987) or more accumulation of metabolic products within shoot or root organs (Ohwaki & Hirata 1992). However, exudation rates were relatively constant when related to dry weight of root. At different plant stages, the exudation rates varied between 18.3–32.0 (14 days), 3.4–5.4 (28 days), 1.4–2.7 (47 days) and 4.7–11.5 (76 days) μmol C h^{-1} $g_{d.w}^{-1}$ and did not show any effect of P treatment. Thus, high exudation

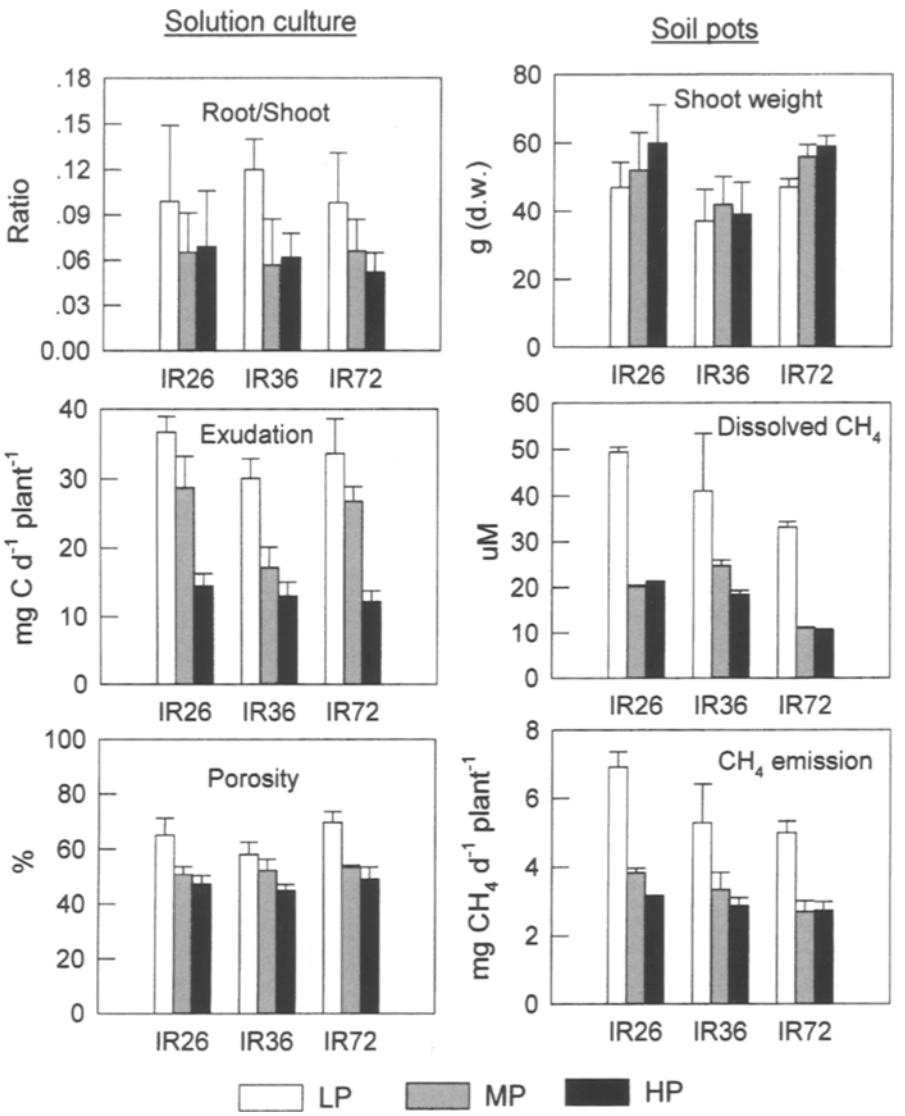

Figure 4. Plant and soil parameters at 76 days of plant age (flowering stage) in solution culture and soil pots with low (LP), medium (MP) and high (HP) P treatments. Bars indicates standard deviation.

rates per plant were caused by larger root biomass and not by higher activity of the root tissue.

Higher exudation is a response by the plant to increase P acquisition under stress condition (Gardner et al. 1983; Ae et al. 1990; Marschner 1996). High excretions of citric and malic acids result in localized acidification of

the rhizosphere which – in turn – mobilizes the sparingly-soluble inorganic compounds such as phosphates, iron, manganese and zinc (Hoffland et al. 1989). However, the increases in root exudates also increase loss of photosynthates from roots to soils. This not only seems to be a high cost in terms of the carbon economy of plants, but also enriches carbon source for methanogenesis in rice soils.

Root porosity increased in response to P deficiency as previously observed by Kirk and Du (1997). The increase in root porosity in low P could promote gas exchange between the atmosphere and the rhizosphere, i.e., the downward transfer of oxygen and upward transfer of methane (Justine & Armstrong 1987; Kludze et al. 1993).

The differences in root exudations and root porosity in response to phosphorus levels have been obtained from culture solution experiments and therefore, may not reflect the conditions occurring in soils. However, corresponding trends observed in shoot biomass in culture solution (Figure 1) and soil culture (Figure 4) indicate similar response patterns by plants. The findings obtained in soil experiments can successfully be explained by results of the solution culture. High values of dissolved methane and methane emission were apparently related to a chain of response mechanisms by the plant to P stress:

(1) higher allocation of assimilates to the roots,
(2) enhanced root exudation, and
(3) increased vertical transfer of methane.

In this study, the values of dissolved methane concentrations and methane emission rates were relatively high during the early stage, while methane emission and dissolved methane concentration became very low after flowering stage. This pattern deviates from findings in field experiments where dissolved methane and methane emission rates were low in the early stage (Wassmann et al. 1994). Schipper and Reddy (1996) suggested that in the pot experiment the relative amount of root biomass and volume within soil-root system increased compared to soil experiment. Therefore, the impact of roots will be amplified as compared to field conditions. The close relationship between methane emission and the pool of dissolved methane in soil solution was previously documented in field experiments (Wassmann et al. 1996). In this study the seasonal pattern of methane emission rates generally followed the dissolved methane concentration in soil solution. At a given stage, different concentrations of dissolved methane also resulted in different emission rates – except for the early stage when gas-transfer capacity of the plants is still very low.

Conclusions

The results of this study demonstrate that P deficiency of rice plants increase CH_4 production and emission. The impacts of P fertilization on methane emission might be strongly related to root growth, root exudation and aerenchyma formation of rice plants. Although the hydroponics studies do not necessarily reflect actual conditions in soil, the study provides a theoretical basis for understanding the influence of root growth and physiology on methane production and emission from rice paddies. The results of this study also suggest that P fertilization can not only improve grain yields but also mitigate methane emission in P-deficient soil. Such mitigation choice with dual benefits should be a complement to global mitigation strategy for CH_4 emission from rice agriculture.

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