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Phosphorous and Iron Reactions as Influenced by pH and Oxygen Released in the Rice (*Oryza sativa*) Rhizosphere

by

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the degree of Doctor of Philosophy

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ABSTRACT

Ph. D. Caroline B. M. Begg Natural Resource Sciences

Lowland rice production is expanding throughout South-East Asia necessarily onto soils of poorer nutrient status with a resulting decrease in yields. An understanding of the nutrient status of the rice rhizosphere is essential for the development of appropriate management practices to increase rice yields. Phosphorus (P) deficiency is one aspect of rice nutrition. Increased rice root respiration and P uptake efficiency, and an increase in H^+ released from roots and enhanced solubility of calcium phosphates are two possible mechanisms of tolerance to low P levels. These mechanisms were evaluated but could not be used as single tests to differentiate among cultivars for tolerance to P deficiency. Phosphorus reactions in the soil may be confounded by the chemistry of iron (Fe). Iron and P interactions in the rice rhizosphere were investigated using a Philippine paddy soil. Root loss of oxygen (O_2) into the rhizosphere caused the oxidation of Fe^{2+} and the concurrent release of H^+. Root release of H^+ from cation-anion uptake imbalances also contributed to the acidification of the rhizosphere. Accumulation of Fe^{3+} was found next to the root plane. Depletion of acid-soluble P coincided with the zone of acidification. Rice plants were able to utilize the acid-soluble P fraction during growth.

RÉSUMÉ

Thèse de doctorat | Caroline B. M. Begg | Département des sciences des ressources naturelles

La culture du riz irrigué occupe des superficies de plus en plus importantes dans la région du sud-ouest asiatique. Ceci mène nécessairement à l'utilisation de sols moins fertiles ce qui entraîne une diminution de rendements. Il est donc essentiel de bien connaître le rôle de la rhizosphère dans la nutrition minérale de cette culture afin de mettre en place des régies appropriées visant à augmenter les rendements de riz.

Les carences en phosphore (P) sont une préoccupation importante dans la nutrition minérale du riz irrigué. L'augmentation de la respiration des racines et de leur efficacité d'absorption du P, ainsi qu'une production accrue de protons (H^+) par les racines, qui solubilise les phosphates calciques, sont deux mécanismes par lesquels cette culture peut tolérer des sols pauvres en P. Ces mécanismes ont été évalués. Utilisés seuls, ils n'ont pas pu distinguer les seuils de tolérance des différents cultivars mis à l'essai.

Les réactions du P dans le sol peuvent être influencées par la présence du fer (Fe). Les interactions entre le fer et le P dans la rhizosphère du riz irrigué ont donc été étudiées dans un sol d'une rizière des Philippines. L'oxygène libéré par les racines dans la rhizosphère a oxidé le Fe^{2+} et contribué à la production de H^+.

La libération de H^+ par les racines suite à l'absorption inégale des cations et des anions, a aussi contribué à l'acidification de la rhizosphère. Le Fe^{3+} s'est accumulé près des racines et l'épuisement en P soluble à l'acide coïncidait avec la zone d'acidification. Les plantes de riz étaient donc en mesure d'utiliser la fraction de P soluble à l'acide du sol pendant leur croissance.

PREFACE

Rice production is rapidly expanding in South-East Asia. To meet increased demands for higher yielding rice cultivars, a greater understanding of the rice root-soil interface and nutrient uptake is required. The purpose of this research was 1) to evaluate root activity of rice cultivars, 2) to investigate the influence of oxygen (O_2) diffusion on iron (Fe) reactions and pH in the rice rhizosphere in anaerobic soils and 3) to evaluate the changes in rhizosphere pH on soil phosphorus (P) fractions and P uptake by rice cultivars.

This thesis comprises five chapters, preceded by a general introduction. Chapter I is a review of current literature, which discusses P and Fe chemistry in anaerobic soils, O_2 diffusion from rice roots and methodology for studying the rhizosphere. Hypotheses to be tested were developed from this review. Chapter II investigates root oxidizing activity and proton (H^+) release from rice roots in the rhizosphere. Chapter III examines the effect of Fe oxidation on pH changes in the rhizosphere. Chapter IV evaluates the effect of rhizosphere pH on P fractions. Chapter V provides some general conclusions and suggestions for future work. The Appendix contains detailed results related to this thesis.

Chapter II through III are presented in paper format, and conform to requirements set by the Faculty of Graduate Studies and Research. The following statement is excerpted from the Guidelines Concerning Thesis Preparation (1991);

The candidate has the option, subject to the approval of the Department, of including as part of the thesis, the text or duplicated published text (see below), of an original paper, or papers.

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Chapters II and IV are prepared as papers and will be submitted to the Plant and Soil journal. Chapters II and IV were co-authored by the candidate and her supervisors Professor A. F. MacKenzie and Drs. G. J. D. Kirk and H.-U. Neue.

The candidate was responsible for preparing the manuscripts in Chapter II and Chapter IV. Supervisory assistance was provided by Professor A. F. MacKenzie and Drs. G. J. D. Kirk and H.-U. Neue through general guidance and editorial correction and comments during the preparation of the manuscripts. Chapter III has been published in New Phytologist, 128: 469-477, 1994. Chapter III was co-authored by the candidate, her supervisors Professor A. F. MacKenzie and Drs. G. J. D. Kirk and H.-U. Neue. Chapter III was written in collaboration with Dr. G. J. D. Kirk. The candidate was responsible for the design of experimental procedures and for conducting all original research.

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CONTRIBUTIONS TO KNOWLEDGE

Iron and P interactions in anaerobic soils within the rice rhizosphere were investigated. Several contributions to knowledge are presented.

1. Two mechanisms of rice tolerance to low P levels were suggested from root activity as measured by α-naphthylamine oxidation and rice root release of H^+ due to an imbalance in the cation-anion uptake ratio. High root respiration (root activity) could indicate efficient metabolism and enhanced uptake of P in P deficient conditions. A decrease in rhizosphere pH could solubilize calcium phosphates. However, each mechanism alone could not be used as a single indicator of tolerance to P deficiency.

2. An apparatus and system for studying the rhizosphere in anaerobic soils was developed. This methodology facilitated the examination of Fe, P, and pH concentration profiles next to a planar root layer through time.

3. Oxygen passively released by rice roots oxidized mobile Fe^{2+} found in anaerobic soils. Upon oxidation, the H^+ produced contributed to acidification of the rice rhizosphere. Steep pH gradients in the soil were found close to the root surface. Ferric iron accumulated within 2 mm of the root surface. This Fe accumulation has major implications for nutrient availability in the rhizosphere and nutrient uptake by rice.

4. An imbalance in the cation-anion uptake ratio of rice in P deficient soils caused acidification of the rhizosphere due to extrusion of H^+ by the roots. The acidification greatly affects nutrient dynamics adjacent to the rice roots.

5. A methodology was developed to study P fractions in anaerobic soils. Transformations of P fractions in the rhizosphere were examined using this methodology. The P fractionation scheme, although empirical, adequately represented the P pools or complexes found in anaerobic soils.

6. Acidification of the rhizosphere caused solubilization of the acid soluble P fraction. This P fraction was depleted in the rhizosphere, and served as the major source of P for rice. Although the acid soluble P fraction forms a small fraction of the total P found in anaerobic soils it is readily available to rice, which may explain the variable response to P fertilization in the field.

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INTRODUCTION

Population growth in South and Southeast Asia has intensified demands for increased rice production. Arable soils under rice (*Oryza sativa* L.) cultivation are being lost due to urbanization. Soil degradation (erosion, salinization etc.) caused by poor management is contributing to a decrease or stagnation of rice yields. Losses of arable land have resulted in increased proportions of low fertility and adverse soils brought under rice production (Neue and Zhong-lin, 1990).

Adverse soils (such as saline, acid sulfate etc.) and some low fertility soils suffer from nutrient toxicities and deficiencies. Phosphorus (P) is regarded as the second most limiting nutrient to rice and upland crop production with nitrogen (N) as the first (Jones et al., 1982; Moller Nielsen et al., 1986). Phosphorous deficiency is found in major soil groups such as the Ultisols, Oxisols, Inceptisols, and Vertisols (Akbar et al., 1986; De Datta, 1981). Low total P, low plant available P or a high P fixation capacity may all contribute to a lack of P for plant growth. Increasing yields and intensified cropping require P fertilizer application even on fertile soils (De Datta, 1988).

Fertilizer application can ameliorate P deficiency symptoms in rice but crop response to applied P is not as easily recognizable as its response to N. This is usually due to insufficient application of P fertilizer, as much of the applied P is fixed on the soil matrix. As the cost of fertilizers increases, farmers in developing countries are less likely to apply sufficient P to their crops. As both P and N are required for plant growth, the constraint of one nutrient will limit rice yields

(Yoshida, 1981). One major impediment to the prediction of P and rice interactions is the lack of knowledge about reactions near the root. Such reactions are complex, involving short term oxidation of a predominantly anaerobic environment (Armstrong and Beckett, 1987; Drew and Stolzy, 1991). Oxidation-reduction effects are observed with solution and mineral Fe compounds (Chen et al., 1980; Marschner, 1991). Such compounds are correlated with P sorption and precipitation reactions (Willett et al., 1978). Consequently, study of the short range effects of oxygen, Fe and P diffusion near the root are necessary to understand and manage the system. Knowledge is lacking due to experimental difficulties associated with anaerobic rhizosphere studies. A better understanding of P chemistry will aid P requirements and efficient fertilizer P management for rice production on adverse and low fertility soils.

Lowland rice requires a minimum concentration of 64 μmol P dm^{-3} in the soil solution for adequate crop growth (Hossner et al., 1973; Jones et al., 1982). Rice cultivars differ in their response to P deficient soils (Fageria et al., 1988; IRRI, 1987; Koyama et al., 1973; Teo et al., 1992). As the amount of P necessary for rice growth is similar among cultivars, differences in P response are mainly caused by the plant's ability to extract P from the soil in the rhizosphere. The interactions between the rice rhizosphere and the soil regarding plant available P has received little study. Phosphorous absorption can be altered by either root morphology (surface area, root hairs etc.) or by the rate at which P reaches the root surface. The latter mechanism is not well understood (Gardner et al., 1983). Knowledge

about the development of the P depletion zone surrounding the roots would furnish information about uptake characteristics and improve model predictions of crop response to P (Kraus et al., 1987).

Elucidation of P kinetics and chemistry at the soil-root interface would lead to better fertilizer application practices and wiser selection criteria for rice cultivars tolerant to P deficiency. This thesis presents information concerning P uptake and dynamic Fe, P and pH relationships in the rice rhizosphere.

CHAPTER I

LITERATURE REVIEW

1. CHEMICAL PROCESSES UPON SUBMERGENCE

The rice plant (*Oryza sativa* L.) is unique as a major cereal crop in that it thrives in submerged soils. Several chemical processes occur upon soil submergence which influence P chemistry.

Upon submergence, oxygen concentrations in the soil are rapidly depleted by aerobic microorganisms. The decrease in oxygen corresponds with a reduction in the oxidation - reduction potential (Eh) of the soil. The magnitude of reduction will depend on the amount of easily oxidizable organic matter and presence of redox couples. Soil-Fe, which helps to stabilize the Eh between +100 and -100 mV (assuming sufficient quantities of soil-Fe) is the most important redox buffer system in paddy soils (Ponnamperuma, 1976).

Reduction of ferric Fe (Fe^{3+}) to ferrous Fe (Fe^{2+}) and oxidation of organic matter increases the concentration of the hydroxyl ion (OH^-) resulting in a rise in pH. Accumulation of CO_2 formed by concurrent organic matter decomposition, and microbial and root respiration, decreases the soil pH through a reaction of CO_2 and H_2O and increased concentration of HCO_3^-. In acid soils pH approaches neutrality due to the reduction of soil-Fe while in alkaline soils the increase in pCO_2 decreases pH. Release of exchangeable Fe^{2+} and of HCO_3^- increases the ionic strength of the soil solution. A freshly submerged soil will not approach equilibrium status for several weeks. For example Fe^{2+} reaches equilibrium concentration values after 12 weeks and depending on the soil type, pH values

Organic matter, as root exudates, green manure (e.g., *Azolla*) or rice straw, provides the energy source for anaerobic microorganisms. Oxidation of organic matter supplies electrons that cause soil reduction. The intensity of reduction will depend on the amount of easily oxidizable organic matter and availability of electron acceptors.

The oxidation-reduction potential of a soil can be characterized by a single redox parameter: pe + pH (Lindsay, 1979). The term pe is defined as the negative log of the electron activity. As the oxidation-reduction reaction always involves both H^+ and e^-, the stability of the solid phases present in the soil depends on the balance of the two values. Representations of pe versus pH show thermodynamic relationships under equilibrium conditions. Conclusions from these representations can be applied with caution to non-equilibrium soil systems as they suggest possible energy relationships between redox species (Bartlett, 1986).

2. SOLID PHASE P

Crystalline forms of inorganic P are unlikely to occur in paddy soils. Redox cycles (oxidation-reduction) induce the dissolution of solid phases and precipitation of compounds generally in an amorphous state with variable composition. Additionally, rates of reaction with crystalline forms are very slow in soil solution. Organic matter, as microbial decomposition products or root exudates, can block the sites of crystal growth (Cornell and Schwertman, 1979). However, examination of thermodynamic diagrams show the possibility of several solid phase P compounds in equilibrium with soil solutions.

In acid soils (pH 3 to 6), vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$), may precipitate under saturated conditions of a low Eh, high solution P and high soluble Fe^{2+} concentrations. As the system oxidizes, vivianite dissolves and strengite ($FePO_4 \cdot 2H_2O$) can form under conditions of high Eh, low pH and a high P concentration (Nriagu, 1972).

Iron in paddy soils is present in more soluble forms compared to aerobic soils due to large amounts of amorphous Fe hydroxides. Strengite could precipitate at lower phosphate ion concentrations, but the phosphate ion reacts preferentially with Fe^{3+} hydroxides present in the system (Hearn et al., 1983). The possibility of this compound occurring in the oxidizing or drying cycle of wetland soils in the presence of highly reactive amorphous iron hydroxides that strongly sorb P is unlikely. Variscite ($AlPO_4 \cdot 2H_2O$) formation can occur in very acid soils (pH < 3) but is unstable at higher pH values which occur upon flooding. Oxidation of the soil will favour strengite over variscite due to the greater stability (lower solubility) of strengite.

As lowland rice soils are subject to continuous cycles of wetting and drying, long term equilibrium between soil solution and solid phases is rarely achieved. Generally the soil is in a metastable state between dissolution and precipitation. Labile P rather than specific crystalline compounds determine the equilibrium concentration of P in soil solution and the rate at which P is supplied after removal by plant roots (Fox, 1986).

3. ORGANIC P

The importance of organic P to rice nutrition is difficult to assess. Uwasawa et al. (1988) observed a low ratio of organic P to total P, although during rice cultivation the organic P content increased. The low organic P level was attributed to the rapid rate of mineralization of organic P under submerged conditions (Lin, 1973; Uwasawa et al., 1988). Phosphorus can complex with organic matter. In one study as the amounts of soluble organic matter increased the content of soluble organic bound phosphorus increased amounting to 70% of the total P in solution (Welp et al., 1983).

4. PHOSPHORUS SORBING SURFACES

Orthophosphate ($H_2PO_4^-$ or HPO_4^{2-}) in soil solution reacts with variable charge and constant charge surfaces. The constant charge surfaces are represented by clay mineral faces with layers of sorbed cations that balance permanent charges created by isomorphous substitution. Variable charge surfaces include, Fe and Al oxides and hydroxides, clay edges and organic matter. Charges are variable because they are dependent on pH values, increasing as pH increases.

Most lowland rice soils are dominated by Fe compounds. Phosphorus reactions with crystalline Fe compounds such as goethite and lepidocrocite are slow. Amorphous Fe hydroxides have a large reactive surface area and a high P sorbing capacity (Willett, 1979). Aging produces crystalline Fe oxides with low sorbing capacities. Conditions of alternating redox produce amorphous Fe hydroxides that exist on the surface of clay minerals as discontinuous coatings.

Oxidation status and intensity of oxidation (length of time) will determine the reactivity of these compounds.

Under reducing conditions the concentration of Fe^{2+} increases. Above pe + pH = 8.5 the precipitation of the mixed valence (Fe^{3+} - Fe^{2+}) ferrosic-hydroxide ($Fe_3(OH)_8$) will occur (Schwab and Lindsay, 1983). Oxidation causes the transformation of $Fe_3(OH)_8$ to a more crystalline state without a dissolution step but the sorption capacity is still high (Holford and Patrick, 1981). Schwab and Lindsay (1983) suggest the mixed valence $Fe(OH)_n$ can persist in environments of changing redox but is dependent on the intensity of oxidation. Precipitation of siderite ($FeCO_3$) occurs below pe + pH = 8.5 but siderite is not stable under oxidized conditions and forms oxides and hydroxides (Schwab and Lindsay, 1983; St Cyr and Crowder, 1988).

These poorly crystalline phases of Fe^{3+} and Fe^{2+} control the solution concentration of Fe^{2+} under alternating redox. As well, their surfaces control, by sorption-desorption reactions, the solution concentration of P. These phases are metastable in the soil system, thus they are difficult to identify and characterize by such devices as solubility diagrams under equilibrium conditions.

5. TRANSFORMATIONS OF P UPON FLOODING

When soils are flooded there is an initial increase in the concentration of solution P (Khalid et al., 1977; Turner and Gilliam, 1976; Willett and Higgins, 1978). The increase in solution P is not permanent. Submergence induces major alterations in both the physical and chemical nature of the soil and these

alterations change soluble P with time.

Upon flooding solution P can be increased by several mechanisms: 1) reduction of Fe^{3+} oxides with the release of surface P and occluded P in the matrix, 2) desorption by exchange of organic anions of P held by clay surfaces and Fe hydroxides, 3) hydrolysis of Fe^{3+} and Al phosphates as the pH rises and the dissolution of Ca phosphates as the pH falls, 4) desorption of P from clay and Fe and Al oxides (De Datta, 1981; Hossner et al., 1973; Ponnamperuma, 1972).

The soil conditions which release P into solution also facilitate P adsorption. Newly precipitated amorphous compounds provide surfaces for P sorption. Additions of readily oxidizable organic matter intensify reduction, increase the concentration of Fe^{2+} and the precipitation of amorphous Fe compounds and Fe^{2+} phosphates. Several studies have shown positive correlations between P sorption and the content of poorly crystalline, ammonium oxalate extractable Fe compounds (Khalid et al., 1977; Kuo and Mikkelsen, 1979).

The influence of organic matter in submerged soils on P sorption is complex. Lower Eh values will promote reduction of Fe^{3+} phosphates and of occluded P within Fe^{3+} oxides and hydroxides increasing concentration of P in solution. Organic acids promote the dissolution of surface-bound Ca, Al and Fe phosphates (Goh et al., 1986). Organic anions will compete for P adsorption sites thus reducing P sorption (Mengel, 1985; Sibanda and Young, 1986). However, low Eh will also facilitate production of amorphous Fe hydroxides increasing P adsorption (Willett et al., 1978). Crystallization of Fe hydroxides is slowed by

organic acids and by a low pH thus maintaining a reactive surface for P (Schwertman, 1966). Willett and Higgins (1978) observed that additions of rice straw compared with no addition accelerated the initial rate of P sorption under flooded conditions but by the end of the incubation period there was no difference in the total amount of P adsorbed.

The buffer capacity of some soils is increased by flooding (Roy and De Datta, 1985; Willett, 1986). Phosphorous sorption at medium to high concentrations of added P is greater in submerged soils than aerobic soils (Patrick and Khalid, 1974; Willett and Higgins, 1978). Alterations in buffer capacity are reflections of changes in soil-Fe. Crystalline forms of Fe hydroxides can bind orthophosphate more firmly than amorphous Fe compounds but their surface area is less. The newly precipitated amorphous Fe compounds have a lower affinity for P but their total capacity is much higher due to a large surface area (Patrick and Khalid, 1974; Khalid et al., 1977). The rise in pH upon flooding decreases the positive charge on the variable charge surfaces and should decrease sorption. Increased ionic strength of the soil solution alters the deposition of ions near the adsorbing surface by suppressing the diffuse layer (Barrow et al., 1980). Depending on the soil, these two reactions will vary in their influence on P sorption. At a pH greater than 5.0, increasing ionic concentration increased P sorption (Barrow and Ellis, 1986).

Flooding and drying cycles tend to increase the P sorption capacity of the soil (Willett, 1982). Kuo and Mikkelsen (1979) observed a decrease in pH and an

increase in amorphous Fe compounds with frequent oxidation-reduction cycles. In some cases re-oxidation of a flooded soil causes an increase in P sorption capacity only if the pH or Eh during reduction is sufficiently low to maintain a high Fe^{2+} concentration (Holford and Patrick, 1981). Many studies have recorded a low solution P and high buffer capacity in re-oxidized soils persisting over long periods (three years) (Sah and Mikkelsen, 1986; Willett and Higgins, 1980).

The increase of solution P concentration upon flooding supplies P to rice but not necessarily to upland crops following rice (Brandon and Mikkelsen, 1979). As all of the reactions described above vary in their intensity with time, the fluctuations of solution P depend on time.

Within the rhizosphere a third factor has to be considered, the effect of the plant root and of its unique microenvironment on P concentration. Examination of the rhizosphere is essential to understand its effect on P reactions. Transformations of P within the rhizosphere are not easily monitored.

6. THE RHIZOSPHERE

Nutrient supply to a plant depends not only on the concentration of an element in the soil solution and its interaction with the solid phase, but on growth, distribution and shape of the roots and on their physiological ability to mobilize and absorb nutrients (Jungk and Claassen, 1986). Examination of the rhizosphere focuses research on an area important to plant uptake of nutrients.

Study of the rice rhizosphere is unique as rice has the ability to re-oxidize the rhizosphere by diffusion of atmospheric oxygen through the aerenchyma into

the rooting zone. Stems and roots of rice plants develop internal gas spaces upon cell death and dissolution which then form the aerenchyma system (Drew and Stolzy, 1991). Oxygen transport from shoot to root is generally considered a simple physical diffusion. The partial pressure of O_2 decreases with an increase in distance between root base and root tip (Armstrong, 1972). As O_2 diffuses through the aerenchyma, a certain portion of O_2 may be consumed by respiring tissues and part may be lost by lateral leakage through cell walls (Armstrong, 1971).

The oxidative zone protects rice from toxic substances such as Fe^{2+} and sulfides (Ando et al., 1983). The reduced Fe precipitates as iron oxides either on the root or on surrounding surfaces (Johnson-Green and Crowder, 1991). Some authors propose that the precipitation of Fe and Mn oxides on root surfaces form a protective shell against Fe^{2+} toxicity (Chen et al., 1980; Kimura et al., 1982). Alternatively, Wright and Hossner (1984) suggest that Fe oxide coatings may reduce subsequent uptake of Fe, Mn and other cations, depending on the degree of coverage. Thick layers of Fe oxides reduce the activity of root hairs (physical inhibition) which in turn reduces the nutrient absorptive capacity of the plant. Depending on age and cultivar, the rice root will vary in its ability to oxidize the rhizosphere. Several authors have reported differences in the O_2 diffusion rates among rice cultivars (Armstrong, 1969; Lai and Hou, 1983; Wright and Hossner, 1984).

Rice root tips are generally white with no discolouration due to Fe oxide

coatings. The O_2 diffusion rate is greatest in the apical region with the oxidation sphere extending several mm into the surrounding soil causing the precipitation of Fe^{2+} as Fe^{3+} away from the root surface (Armstrong, 1967; Jensen et al., 1967). Root tips and young rice roots exhibit a greater oxidizing power but this ability of the roots decreases as the plant matures (Green and Etherington, 1977; Kumazawa, 1984). Kimura et al. (1982) observed that older roots exhibited reducing conditions causing the precipitated Fe oxide coatings to dissolve. High microbial activity (respiration) also can cause the dissolution of Fe^{3+} coatings, increasing the content of Fe^{2+} in solution (Benckiser et al., 1984).

The rhizosphere is not characterized by a single Eh reading but exists in a mixed state of oxidative and reductive potentials depending on O_2 diffusion, moisture content, and root and microbial respiration. Flessa and Fischer (1992) measured increased Eh values in the rice rhizosphere extending 1 mm to 4 mm from the root surface in anaerobic soils. Changes in redox will alter the availability of P in the rhizosphere. The release of P in the bulk saturated soil by reduction of Fe^{3+} phosphates and of occluded or adsorbed P on Fe^{3+} oxides and hydroxides is considered the major mechanism providing P for plant uptake. The oxidative zone of the rhizosphere induces precipitation of amorphous Fe oxides providing ready surfaces for P sorption. Although the saturated condition increases the diffusion coefficient of P (Mouat and Nes, 1986) thus improving P supply to plants, the presence of strongly sorptive Fe oxides increases the P buffer power of the soil, reducing diffusion and decreasing P availability. Consequently,

the rice root competes with the Fe oxides in the rhizosphere for the phosphate ion. As well, root exudates and organic anions will alter the adsorbing surfaces and influence P sorption. Differences of rice cultivars in response to P deficiency may be related to the oxidative ability of the rice root. Knowledge of P reactions in the bulk soil does not explain the rice plant's ability to extract P under conditions imposed by the rhizosphere.

Besides the changes in rhizosphere Eh, the pH within the rhizosphere is also subject to alterations. Nye (1981) illustrated by modelling soil and root processes that if plants absorb N predominantly as NO_3^- the pH of the rhizosphere will rise, while if NH_4^+ is absorbed the pH will fall. The pH at the root surface can differ from the bulk soil a few mm away by one to two units. Absorption of NH_4^+ is correlated with an increased cation uptake and subsequent higher net excretion rate of H^+ (Marschner et al., 1982). The change in the uptake ratio of cations to anions needed to maintain the charge balance causes alterations in rhizosphere pH (Hedley et al., 1982a; Marschner et al., 1982).

Several authors have recorded a decrease in rhizosphere pH (Ahad, 1993; Hauter and Mengel, 1988; Schaller, 1987). The H^+ excretion is not uniform over the entire root but concentrated in the apical regions (initial 2 cm) and root hairs (Moorby et al., 1988; Romheld et al., 1984; Schaller and Fischer, 1985). pH decreases recorded at the root surface and within the rhizosphere will depend on the pH buffering capacity of the soil. Hauter and Mengel (1988) recorded a pH depression of one unit up to 0.5 mm from the root surface on a highly buffered

soil. Schaller and Fischer (1985) recorded a decreased pH up to 2.5 mm in a weakly buffered soil. Romheld et al. (1984) suggest that strong acidification of a small volume of soil in the rhizosphere is possible even in soils of a high pH buffering capacity. Acidification of the rhizosphere depends on plant species, age, form, level of nutrient supply and the buffer capacity of the soil (Romheld et al., 1984).

A decrease in pH can enhance the solubilization of nutrients in the rhizosphere (Marschner, 1991). Acidification of the soil solution from pH 5.8 to 3.9 by maize caused a three-fold rise in the concentration of P (Kraus et al., 1987). The depletion zone for P around the root cylinder was 2 mm for maize and 2.6 mm for rape (Hendriks et al., 1981). Grinsted et al. (1982) recorded a pH drop of 2.4 units with a corresponding increase in the concentration of P in the rhizosphere of rape seedlings. In the same study of rape seedlings, Hedley et al. (1982b) observed a decrease in the acid soluble (non-exchangeable) forms of inorganic P and residual P.

Moorby et al. (1988) studied P depletion in rape. Initially, acid production was only in the apical region but as the seedling became progressively more P deficient H^+ extrusion extended basally 6 cm from the tip. Although P deficiency was associated with H^+ efflux, the release of H^+ did not occur until three days later. Acid production was a response to changes in the metabolism of the plant, not to a decrease in anion uptake.

The mechanisms described above are for aerobic plants. Study of P kinetics

in the rhizosphere of rice plants has received little attention for a number of reasons. The methodology for rhizosphere studies in which nutrient transformations occur within 1 cm or less from the root surface has not been established for flooded anaerobic soils. The importance of P for sustaining rice cropping especially on infertile and adverse soils has been documented only recently. The likely impact of rhizosphere characteristics is now recognized as important for breeding soil stress tolerant rice cultivars.

7. METHODOLOGY FOR STUDY OF RHIZOSPHERE

Several methods have been proposed for the study of the rhizosphere zone inclusive of soil sampling. The rhizosphere is best described as the volume of soil surrounding the root influenced or altered by the physiological characteristics of the root. Most often this refers to a zone that extends only 1 to 3 mm from the root surface. Root and root hair growth can interfere with soil sampling of the rhizosphere. Most methods attempt to minimize the effects of working in a minute region, with live plants, in a poorly defined environment. Success is limited in these studies.

Grinsted et al. (1982) filled a thin section of soil with live roots. The entire section was considered rhizosphere soil after competition was detected between the roots. This method is restricted to a comparison between non-rhizosphere and rhizosphere soil. No study of the nutrient depletion zone is possible.

Helal and Sauerbeck (1983) segregated the soil volume into zones using a nylon mesh that allowed root hair penetration. The innermost zone, next to the

roots, was considered the rhizosphere. Only gross measurements of alterations in nutrients were possible as most nutrient transformations occurred within 3 mm of the root.

Jungk and Claassen (1986) examined P availability in thin sections obtained below the root plane rather than parallel to the vertical root growth. The depletion of P and K was measured in thin sections from soil separated from the root plane by a nylon mesh. In this method only root tips and root hairs were active in P uptake.

Bagshaw et al. (1972b) measured the diffusion of ions in two soil blocks separated by a root plane. Sequential microtome sections were sliced parallel to the vertical root plane. Root tips were allowed to extend into a water tray. Onion roots exhibited little lateral growth while rye grass root hairs were damaged. However, P uptake was not significantly affected.

Hedley et al. (1982b) examined phosphate fractions in the rhizosphere that suffered depletion due to plant uptake. The P fractionation procedure was used to characterize possible changes in the rhizosphere. McLaughlin et al. (1987) suggest that recent developments in the procedures for fractioning soil P have allowed a better understanding of P transformations. A similar procedure with modifications for anaerobic soils could be used to evaluate the ability of rice to extract P either from labile forms or from sparingly soluble compounds.

8. HYPOTHESES

As the result of this review, it is indicated that the effect of rice roots on P and Fe chemistry in the rhizosphere remains to be determined. Thus the following hypotheses are proposed:

1.0 Rice cultivars differ in their response to P deficiency.

1.1 The oxidation of alpha-naphthylamine reflects root activity and could be a measure of cultivar response to P deficiency.

2.0 Oxidation of the rhizosphere by rice roots effects changes in pH and Fe composition.

2.1 The pH next to the rice root drops as a result of Fe^{2+} oxidation and release of H^+ from roots.

2.2 Ferric Fe concentrations peak adjacent to the root surface and decline as the distance increases from the root surface due to O_2 diffusion from the root and consumption in the rhizosphere.

2.3 Ferrous Fe concentrations in soil solution decline near the root surface and increase as the distance increases from the root surface due to O_2 diffusion and consumption in the rhizosphere.

2.4 The total amount of Fe^{2+} oxidized in the rhizosphere (lost from solution) should equal the total quantity of Fe^{3+} produced.

2.5 The total quantity of H^+ found in the soil solution should equal the sum of H^+ produced in Fe^{2+} oxidation and H^+ released by the roots to balance cation uptake.

3.0 The forms and reactions of Fe and P in the rhizosphere differ from those in the bulk soil.

3.1 The reduced bulk soil would exhibit high pH, high concentrations of soluble Fe^{2+} and low quantities of adsorbed Fe^{3+}, while in the rhizosphere these parameters would be reversed.

3.2 The concentrations of acid soluble P would decrease and of alkali soluble P increase in the rhizosphere due to changes in pH, while the reverse would be true in the bulk soil.

3.3 Changes in P fractions would be related to acidification caused by rice roots and by Fe^{2+} oxidation.

9. OBJECTIVES

The objectives of this research were to determine the effects of O_2 diffusion and pH in anaerobic soils on the forms and reactions of Fe and P in the rice rhizosphere. The methodology and experiments were developed to study dynamic changes in pH, Fe reduction and oxidation, and P fraction solubilization and fixation at the soil-root interface in anaerobic soils.

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CHAPTER II

ROOT OXIDIZING ACTIVITY AND ROOT pH OF FOUR RICE CULTIVARS AS INDICATORS OF TOLERANCE TO LOW PHOSPHOROUS LEVELS

1. ABSTRACT

Selection of rice cultivars for tolerance to phosphorous (P) deficiency is important as less optimal rice producing soils with lower P levels are brought under cultivation in South-east Asia. A greenhouse experiment was conducted with four rice cultivars to evaluate root oxidizing activity and root H^+ release (root pH) as indicators of cultivar tolerance to low P levels. Cultivars IR26, IR36, IR7790 and IR9764 were grown in nutrient solutions with two P levels, 16 and 322 μmol P dm^{-3} for 30 days. Quantitative measurements of root pH were obtained from agar mediums containing roots and a pH indicator. Oxidation of α-naphthylamine by intact roots was used to determine the root oxidizing ability of rice cultivars. Root oxidizing activity declined for all cultivars throughout the experiment due to the decrease in the proportion of active roots to total root mass. Differences among cultivars in root oxidizing activity were confounded with interactions between cultivar and P level. Cultivars IR26 and IR9764, both considered tolerant to P deficiencies had the lowest and highest root oxidizing activity, respectively. Root pH at the low P level exhibited the largest decrease in pH for all cultivars compared to high P. IR26 had the smallest decrease in root pH of all the cultivars at the low P level. From the results, neither root pH nor root oxidizing ability can be used alone to differentiate among rice cultivars for tolerance to P deficiency in soils.

2. INTRODUCTION

The rhizosphere refers to a zone that extends about 2 cm from the root surface into the bulk soil (Marschner, 1991). Decreases in pH or increases in oxygen (O_2) content in the rhizosphere, as effected by rice (*Oryza sativa* L.) roots, may change the availability of nutrients due to changes in solid-solution chemistry.

The partially oxidized rhizosphere in rice results from the passive diffusion of O_2 from the roots. Stems and roots of rice plants develop, by cell death and dissolution, internal gas spaces which then form the aerenchyma (Drew and Stolzy, 1991). As O_2 moves from the shoots to the roots via the aerenchyma a certain portion of O_2 may be consumed by respiring tissues and part may be lost by lateral leakage through cell walls (Armstrong, 1971). High rates of O_2 release occur at root tips (Armstrong, 1979). Bedford et al., (1991) found that most of the O_2 transported by shoot to root was consumed by respiration within the plant or by rhizosphere microbes.

Rice roots can oxidize pigments such as α-naphthylamine, which is only slowly oxidized by molecular oxygen but readily oxidized by peroxidase in the presence of hydrogen peroxide (H_2O_2) (Yoshida, 1981). Oxidation of α-naphthylamine is controlled by the amount of H_2O_2, thus the test measures the roots' ability to produce H_2O_2. The enzymatic oxidation of α-naphthylamine (root-oxidizing activity) is positively correlated with root respiratory rate which is an indication of metabolic activity (Ando et al., 1983; Yoshida, 1981). Decreased root-

oxidizing activity can result in decreased nutrient uptake (Dai et al., 1992). Results indicate that young roots oxidize α-naphthylamine at a more rapid rate than old roots (Kawata and Ishihara, 1964).

Alterations in pH within the rice root rhizosphere are due to two processes. Changes in the amounts of H^+ exudation from roots are due to increased cation uptake and a response to external stresses (Marschner, 1991). Trolldenier (1992) observed the dissolution of calcium phosphate in the rice rhizosphere by H^+ extruded by roots. Additionally, O_2 diffusing from the rice roots can oxidize ferrous Fe in an anaerobic environment that will result in a release of acidity during the process (Kirk et al., 1993). Rice cultivars, in their response to environmental stress such as P deficiency or Fe toxicity (high concentrations of Fe^{2+} are released into the soil solution upon reduction during flooding of rice soils), exhibit variation in growth and yield (Fageria et al., 1988; Johnson-Green and Crowder, 1991).

As field trials are expensive and time consuming, a laboratory or greenhouse test of rice tolerance to P deficiency could be beneficial in breeding programs. The present study was undertaken to determine if root-oxidizing activity and root pH differences resulting from H^+ exudation could be used to differentiate among cultivar responses to P deficiency, thus aiding in the selection of rice cultivars for P deficient soils or tailoring P fertilization to cultivars.

3. MATERIALS AND METHODS

Four rice cultivars were selected for study: IR26 tolerant to P deficiency;

IR9764, tolerant to P deficiency and multiple stress; IR36, moderately susceptible to P deficiency but tolerant to multiple stress; and IR7790, susceptible to P deficiency (Lantin, 1989, personal communication).

1). Measurement of plant growth and root-oxidizing activity

Seedlings of each cultivar were grown on Styrofoam boards with individual 5 mm mesh net covered holes (one seedling, one hole) floating in plastic containers filled nutrient solution (Table 1) (Yoshida et al., 1976) at two P levels: 16 μmol P dm^{-3} and 322 μmol P dm^{-3}. pH values of nutrient solutions were adjusted to 5.0 daily for optimum growth, using 1.0 mol dm^{-3} solutions of NaOH and HCl. Nutrient solutions were changed at weekly intervals as suggested by Yoshida et al. (1976). Aeration was maintained by daily stirring for pH adjustment and by weekly change of solution.

Ambient air temperatures in the greenhouse ranged from 25°C at night to 35°C during the day. Relative humidity varied between 60% daily and 90% at night.

Measurements of plant height, root length, root dry matter and α-naphthylamine oxidation were taken at 5 d periods to 30 d. Plant roots were oven dried at 80°C for 48 h to obtain dry matter values.

Analysis for α-naphthylamine oxidation followed that of Ando et al. (1983) and Lai and Hou (1983). Roots of intact rice plants (shoot plus root) were immersed in 50 cm^3 of a 20 mg dm^{-3} solution of α-naphthylamine for 600 s to exclude initial rapid absorption of α-naphthylamine by roots. Subsequently the

roots were transferred to another 50 cm^3 of a 20 mg dm^{-3} solution of α-naphthylamine. Beakers containing the roots were covered with tinfoil to reduce α-naphthylamine degradation from sunlight. A 2 cm^3 sample was taken of each solution at 2, 4 and 24 h. Non-oxidized α-naphthylamine reacted with 50 mg dm^{-3} $NaNO_2$ and 0.1% sulfanilic acid. Absorbance was read at 530 nm with the spectrophometer. Oxidized α-naphthylamine was calculated as the difference between the 20 mg dm^{-3} solution of α-naphthylamine and that in the sample. Plants were sacrificed for the analyses.

2). Measurement of pH along intact roots

The four rice cultivars were grown in nutrient solutions at the two P levels in the greenhouse as described previously. However, in this experiment nutrient solutions were changed daily. Sampling started at four days (after seeding) and continued at single day intervals for two weeks.

The method followed that of Marschner et al. (1982). Intact roots (shoot plus root) were placed in petri dishes (14 cm diameter) with an agar medium (agar concentration = 0.5%) containing one of the two nutrient solutions and a pH indicator, bromocresol purple. Agar and roots were covered with tinfoil. Measurements of rhizosphere pH began after 10 h. Visual observations indicated a decrease in pH by a change in colour, from red to yellow, of the agar medium. Quantitative measurements of the pH change were recorded using a LAZAR PHR-146 Micro combination electrode, tip diameter 1 mm. pH was measured next to six root tips and an average taken of the values. The change in pH was

calculated as the difference between the agar medium pH and the pH adjacent to the root tip. Plants were sacrificed for the analyses.

3). Statistics

Measurement of root-oxidizing activity used a factorial arrangement in a randomized complete block design with four replications. Sample times were included as part of the factorial. As growth patterns of each cultivar were expected to be different through time, statistical analyses were performed on oxidizing activity and plant measurements for each sample period. The root pH study was a randomized complete block with two replicates and an analysis of variance used to find differences between treatments.

4. RESULTS

1). Plant growth

Root length, root dry matter and shoot height significantly increased ($P < 0.01$ level) through the sampling period for all cultivars at both P levels (Figures 1 to 3).

Root length was different ($P < 0.01$ level) among cultivars except at 5 d. Maximum length attained at 30 d was 30.3 cm for IR9764 and a minimum of 23.1 cm for IR26 (Figure 1). Phosphorus level had an effect ($P < 0.05$ level) on root length only on 5 d and 20 d. A cultivar by P level interaction was significant ($P < 0.05$ level) on 15, 20 and 30 d (Table 1). Cultivar IR26, tolerant to P deficiency, had a significantly ($P < 0.05$) longer root length in the low P solution compared to the high P level at 30 d but at 15 d and 20 d there was no significant difference

in root length. Cultivar IR9764, also tolerant to P deficiency, showed no significant difference in root length between P solutions at 15, 20 and 30 days of growth (Table 3). The two cultivars susceptible to P deficiency showed different trends in root length at the two P levels. Cultivar IR36 at 15 d and 20 d, had a longer root length in the low P solution, but on 30 d the longest root length was obtained in the high P solution. Cultivar IR7790 at 15, 20 and 30 d, had the shortest root length in the low P solution.

Root dry mass displayed two patterns during the experiment. From 5 d to 15 d, inclusive, cultivars had a significant ($P < 0.05$ level) effect on the root dry mass with no effect of P. From 20 d to 30 d, inclusive, the 322 μmol P dm^{-3} increased ($P < 0.01$ level) root dry mass with no effect due to cultivars (Table 1). Maximum root dry mass, 668 g, was obtained by IR9764 in the 322 μmol P dm^{-3} solution at 30 d (Figure 2). IR26 had the lowest root dry mass of all cultivars at 30 d at both P levels and the lowest root dry mass during the sample period (Figure 2). IR26 consistently showed the least difference between P levels throughout the experiment.

Shoot height was shorter ($P < 0.01$ level) in the low P solution compared to the high P solution. Significant differences ($P < 0.01$ level) were found among cultivars for all sample periods except at 5 d (Table 1). At 30 d, IR7790 and IR9764 had the greatest shoot height, 58.1 cm and 49.6 cm, respectively, at the 16 μmol P dm^{-3} level (Figure 3). At the 322 μmol P dm^{-3} level, IR7790 had the greatest height, 80.8 cm, ($P < 0.05$ level). Both IR26 and IR36 were shorter than the other

two cultivars at both P levels.

Shoot length to root length ratio decreased ($P < 0.01$ level) in the low P solution for the entire sample period. Cultivars were different ($P < 0.01$ level) from 10 d to 30 d inclusive. Except for cultivar IR36, which is susceptible to low P and tolerant to stress, the ratio increased in the 322 μmol P dm^{-3} level (Figure 4). The shoot: root ratio of IR36 in 322 μmol P dm^{-3} decreased after 15 d and at 30 d the ratio at both P levels was similar.

2). Root oxidizing activity

Discolouration of root surfaces, caused by oxidation of α-naphthylamine, was observed beyond 5 mm from the apical tip that remained white. Lack of colouration on the root tip was probably due to low production of hydrogen peroxide for the enzymatic oxidation of α-naphthylamine.

After 20 d discolouration of the roots was visible on the young lateral roots but not on the mature roots. In the 16 μmol P dm^{-3} solution, root lengths up to 5 mm beyond the apical tips of all cultivars remained white, while in the 322 μmol P dm^{-3} solution the white apical region was generally 2 mm or less.

Significant ($P < 0.05$ level) differences among cultivars in α-naphthylamine oxidation rates occurred from 10 d to 30 d inclusive (Table 2). Differences in oxidation rates due to P occurred on 5 d and 10 d, however, significant ($P < 0.05$ level) cultivar by P interaction occurred from 15 d to 30 d, inclusive (Table 2). Rates of α-naphthylamine oxidation decreased ($P < 0.01$ level) from 2 h to 24 h.

Root oxidizing activity decreased with plant development (Figures 5 and

6). The highest oxidation rate of 9.41 mg α-naphthylamine g dry root^{-1} h^{-1} was obtained by IR9764 at 5 d in the 322 μmol P dm^{-3} solution at the two h sampling (Figure 5). At the 24 h sample time, cultivar IR26 had the highest α-naphthylamine oxidation rate, except 5 d, while IR9764 had the lowest rate at both P levels (Figure 6). Regressions of root dry matter with oxidizing activity were not significant.

3). Measurements of pH along intact roots

Plants from the 322 μmol P dm^{-3} solution showed some loss of turgor while embedded in the agar medium but not plants from the 16 μmol P dm^{-3} solution. This was probably due to the greater demand for moisture as the shoot length was longer in the 322 μmol P dm^{-3} solution. Plants from both P levels were still viable after 10 h in the agar medium.

The results from this experiment measure the root release of H^+ due to imbalances in the cation-anion uptake ratio. Diffusion of H^+, as indicated by the colour change from red to yellow, for all cultivars, was furthest in the 16 μmol P dm^{-3} solution. The leading edge of the yellow colour was observed 4 cm from the root compared to 1.5 cm in the 322 μmol P dm^{-3} solution.

Differences in pH change among cultivars and between P levels were significantly different ($P < 0.01$ level) (Figure 7). There was a significant ($P < 0.01$ level) cultivar by P level interaction. Mean root pH of IR26, from 4 to 8 d, decreased at the low P level, while at the high P level, root pH increased. For all other cultivars, mean root pH declined at both P levels with the smallest decrease

from IR9764 at both levels. From 9 to 14 d, mean root pH decreased at both P levels for all cultivars with the greatest drop in the low P solution. IR26 had the smallest pH decrease at both levels, while both IR36 and IR7790 had the largest pH decrease of the four cultivars at the low P level. The large pH decreases at 7 d was possibly caused by an increase in solar radiation, the result of a clear day, as during the other sample periods the sky was cloudy.

5. DISCUSSION AND CONCLUSIONS

The root oxidizing activity (α-naphthylamine oxidation rate) for all cultivars declined through the sample period due to the decrease in the proportion of active oxidizing root to the total root mass. Except at 5 d, cultivar IR26 had the highest oxidation rate at 24 h in both P solutions and generally the lowest total root mass throughout the experiment. A high oxidizing activity indicated high respiration rate or high metabolic activity that is closely linked to plant growth (Yoshida, 1981).

The greater root length of IR26 in 16 μmol P dm^{-3} than in 322 μmol P dm^{-3} may have been in response to low P levels. A longer root system could exploit a larger volume of soil for P sources. IR26 is considered tolerant of P deficiency and the high respiration rate in the low P level indicated that metabolic activity was not impaired.

Although IR9764 had the lowest oxidizing capacity at 24 h at both P levels, this cultivar is considered tolerant of P deficiency and multiple stress. While root length was the same for both P levels, root mass was the second largest in the 16

μmol P dm^{-3} solution and the largest at 322 μmol P dm^{-3}. For this cultivar the α-naphthylamine oxidation rate is not indicative of its response to low P levels.

Cultivars IR36 and IR7790, which are susceptible to P deficiency showed similar trends in α-naphthylamine oxidation with rates between those of IR26 and IR9764 at 24 h.

Diffusion of H^+ extruded by the roots into the agar medium confounded the measurements of root pH. The largest pH decrease originating from the roots occurred in the 16 μmol P dm^{-3} solution. Thus the actual drop in pH may have been greater near the root tips than was recorded at the low P level. Steep pH gradients (0 to 4 mm from the root surface) are found surrounding rice roots in flooded soils due to the low net rate of H^+ diffusion into the soil near pH 5.3 (Kirk et al., 1993; Nye, 1986).

All cultivars in the low P solution generally showed a decrease in root pH from 7 d to 14 d. The experiment measured H^+ extrusion from roots due to an imbalance in cation-anion uptake. Cultivar IR26, which is tolerant to low P levels in the soil had the smallest pH differential between the two P solutions, which may indicate a greater efficiency of P metabolism within the plant or greater efficiency of P uptake from solution. The two cultivars susceptible to P deficiency, IR36 and IR7790, had the largest pH drop in the low P solution, indicative of imbalances in the cation-anion uptake ratio.

Increased root respiration and an increase in H^+ extruded from the roots at low P levels could indicate an ability of certain cultivars to tolerate P

deficiencies in the soil. Two possible mechanisms of tolerance are suggested. A high respiration rate at low P could indicate efficient metabolism and adequate uptake of P (Dai et al., 1992). A decrease in rhizosphere pH could solubilize calcium phosphates (Trolldenier 1992). However, as the two cultivars tolerant to low P, IR26 and IR9764, showed differing respiration rates (α-naphthylamine oxidation rates) and differing amounts of H^+ extruded from roots, other physiological variables may be involved. α-naphthylamine oxidation rates cannot be used as a single indicator in the selection of rice cultivars tolerant to P deficiency.

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Figure 1. Root length for cultivars in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of four values.

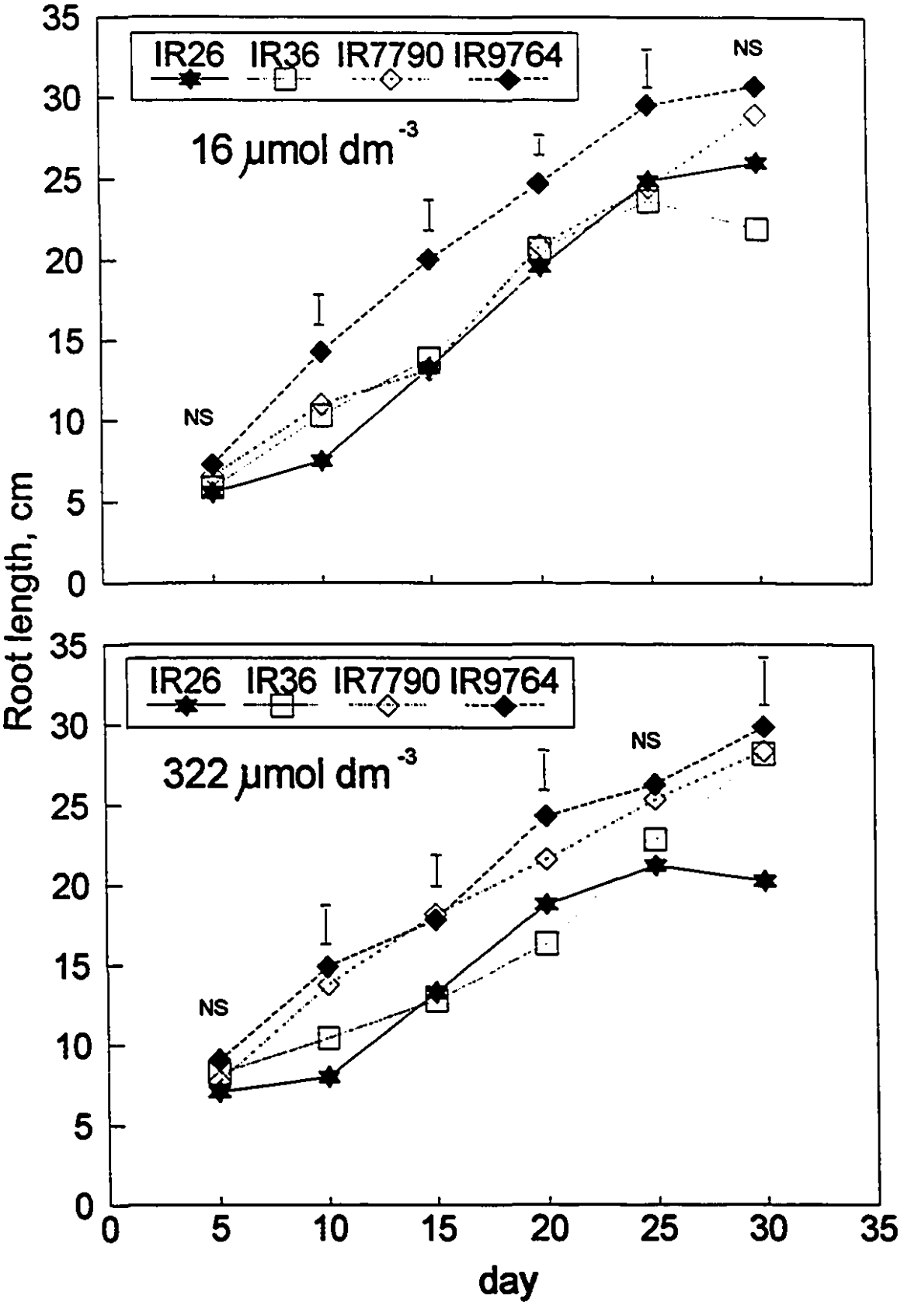
IR26 IR36 IR7790 IR9764
16 µmol dm -3
322 µmol dm -3
NS
NS
NS
NS
Root length, cm
day
0
5
10
15
20
25
30
35

Figure 2. Root dry matter for cultivars in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of four values.

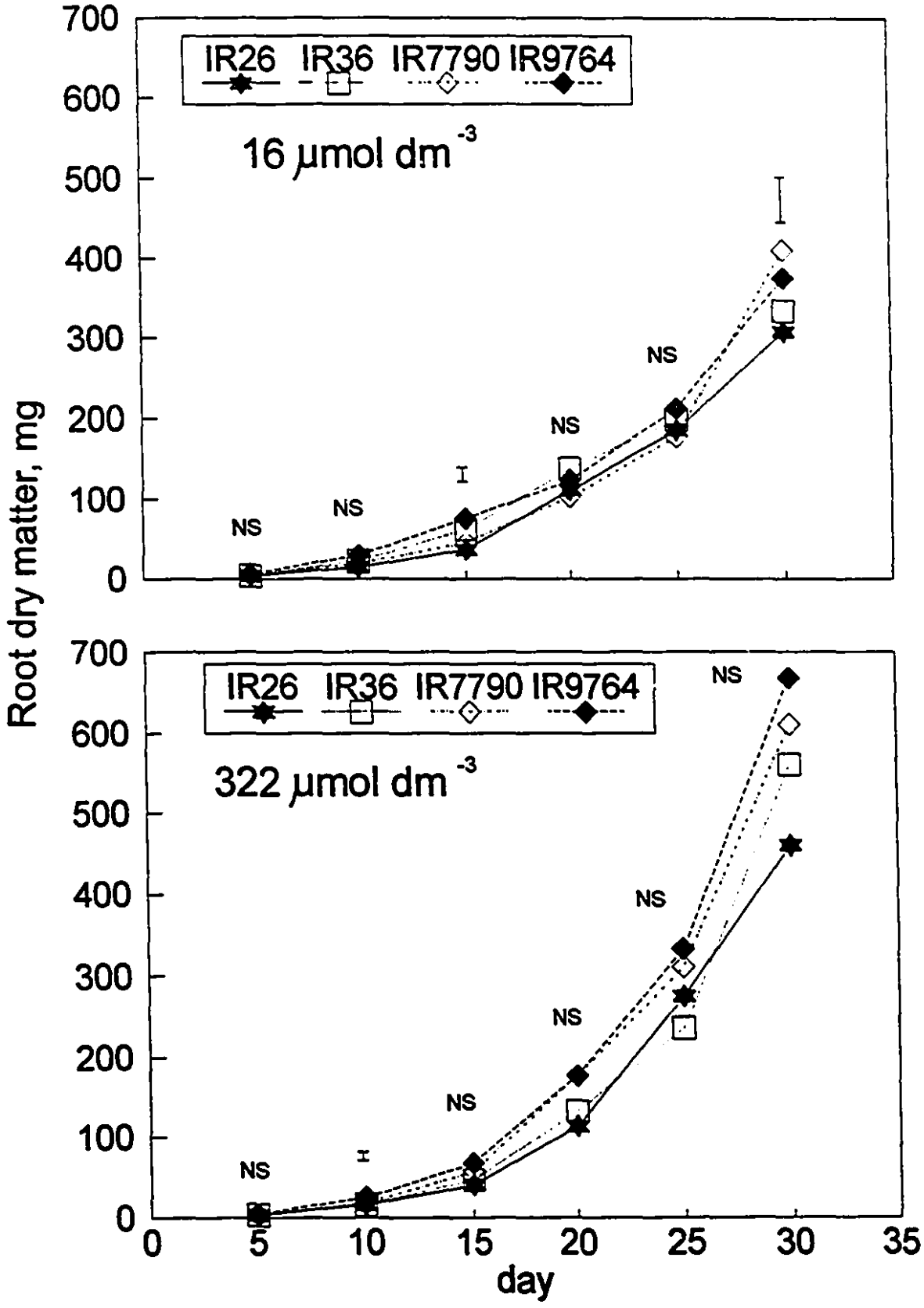
IR26 IR36 IR7790 IR9764
16 µmol dm -3
322 µmol dm -3
Root dry matter, mg
day
NS
0
100
200
300
400
500
600
700
0
5
10
15
20
25
30
35

Figure 3. Shoot length for cultivars in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of four values.

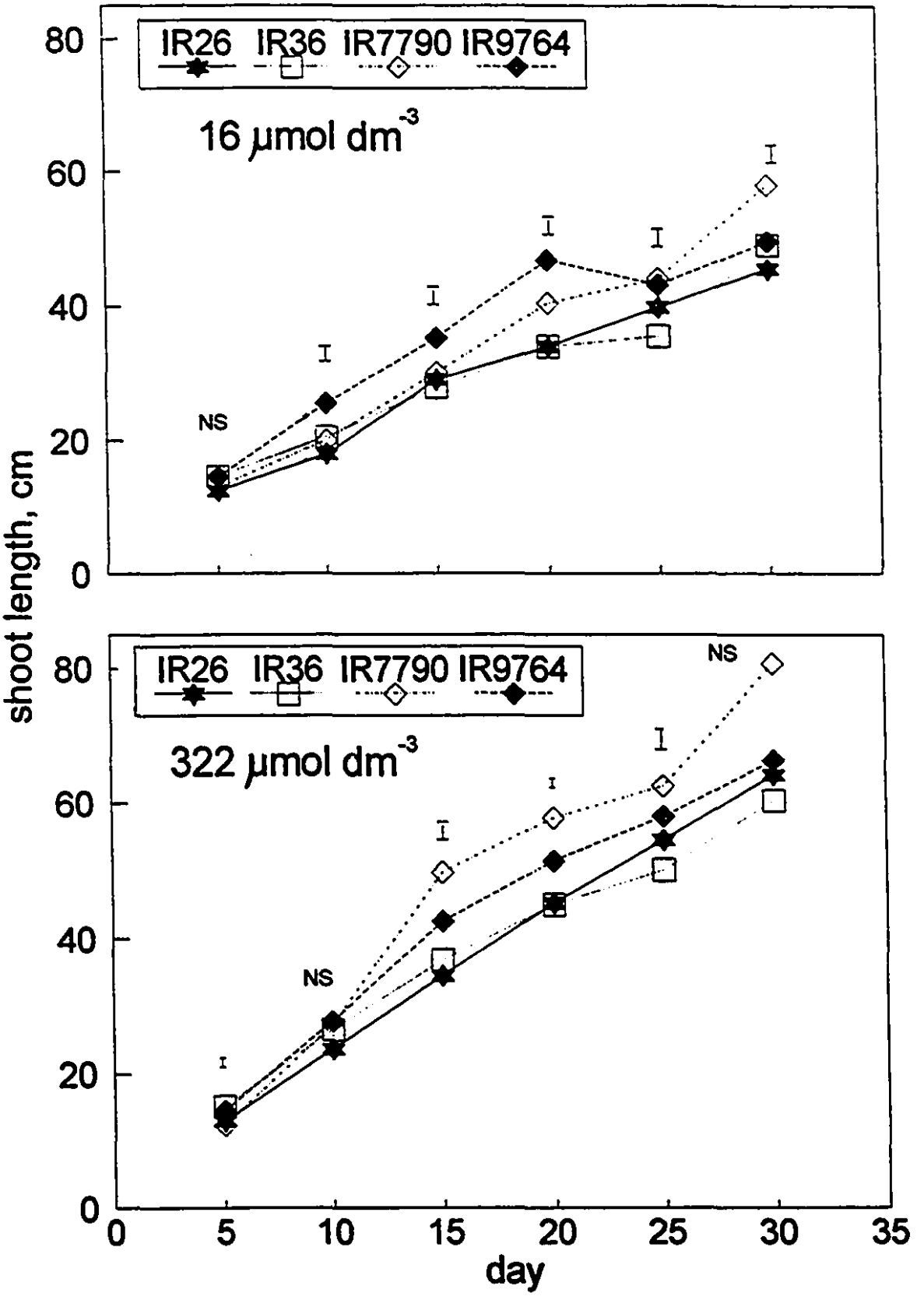
IR26 IR36 IR7790 IR9764
16 µmol dm-3
322 µmol dm-3
shoot length, cm
day
NS
0
20
40
60
80
0
5
10
15
20
25
30
35

Figure 4. Shoot length: root length ratio for cultivars in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of four values.

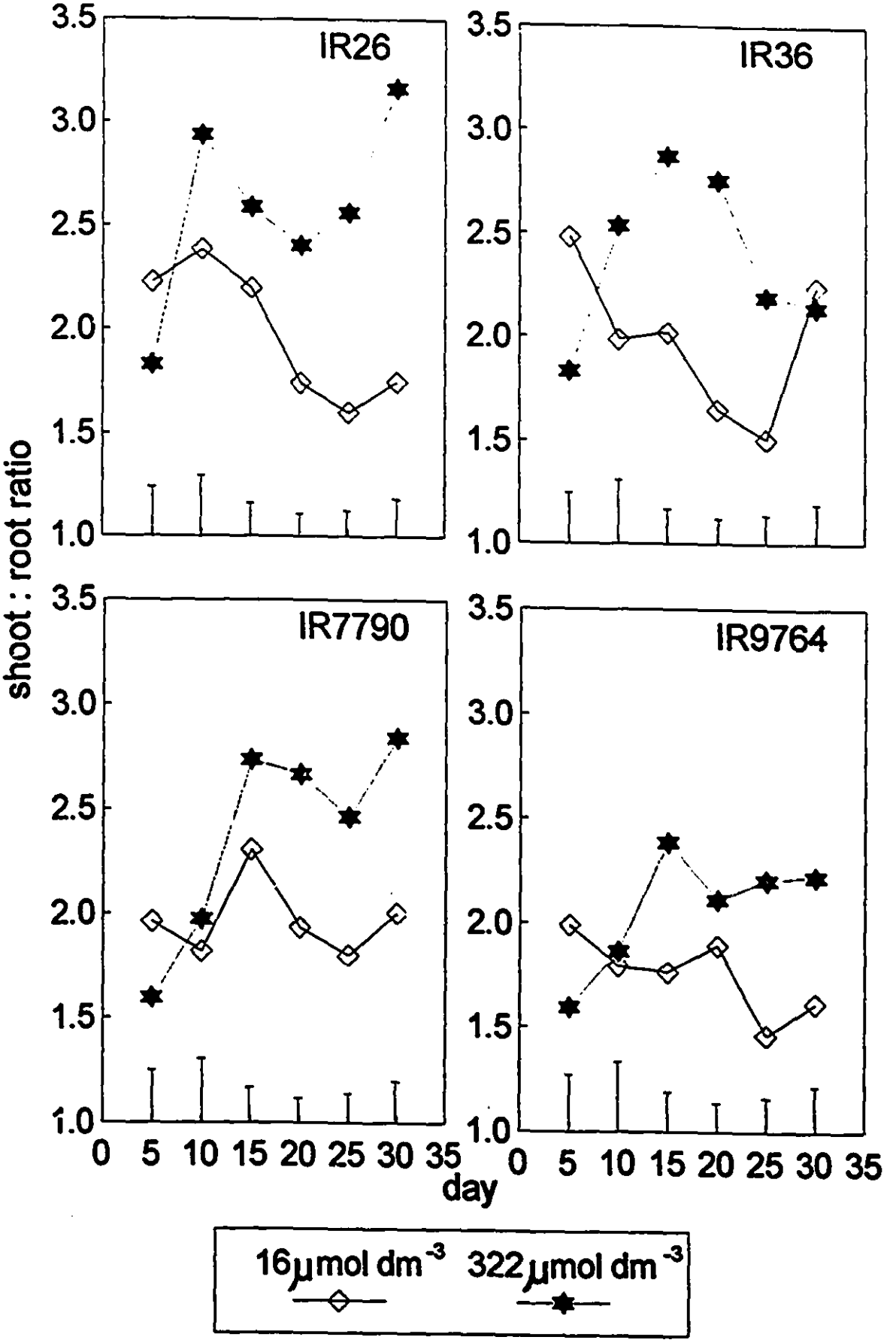
IR26
IR36
IR7790
IR9764
shoot : root ratio
day
3.5
3.0
2.5
2.0
1.5
1.0
0 5 10 15 20 25 30 35
16μmol dm-3
322μmol dm-3

Figure 5. The two-hour α-naphthylamine oxidation rate in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of four values.

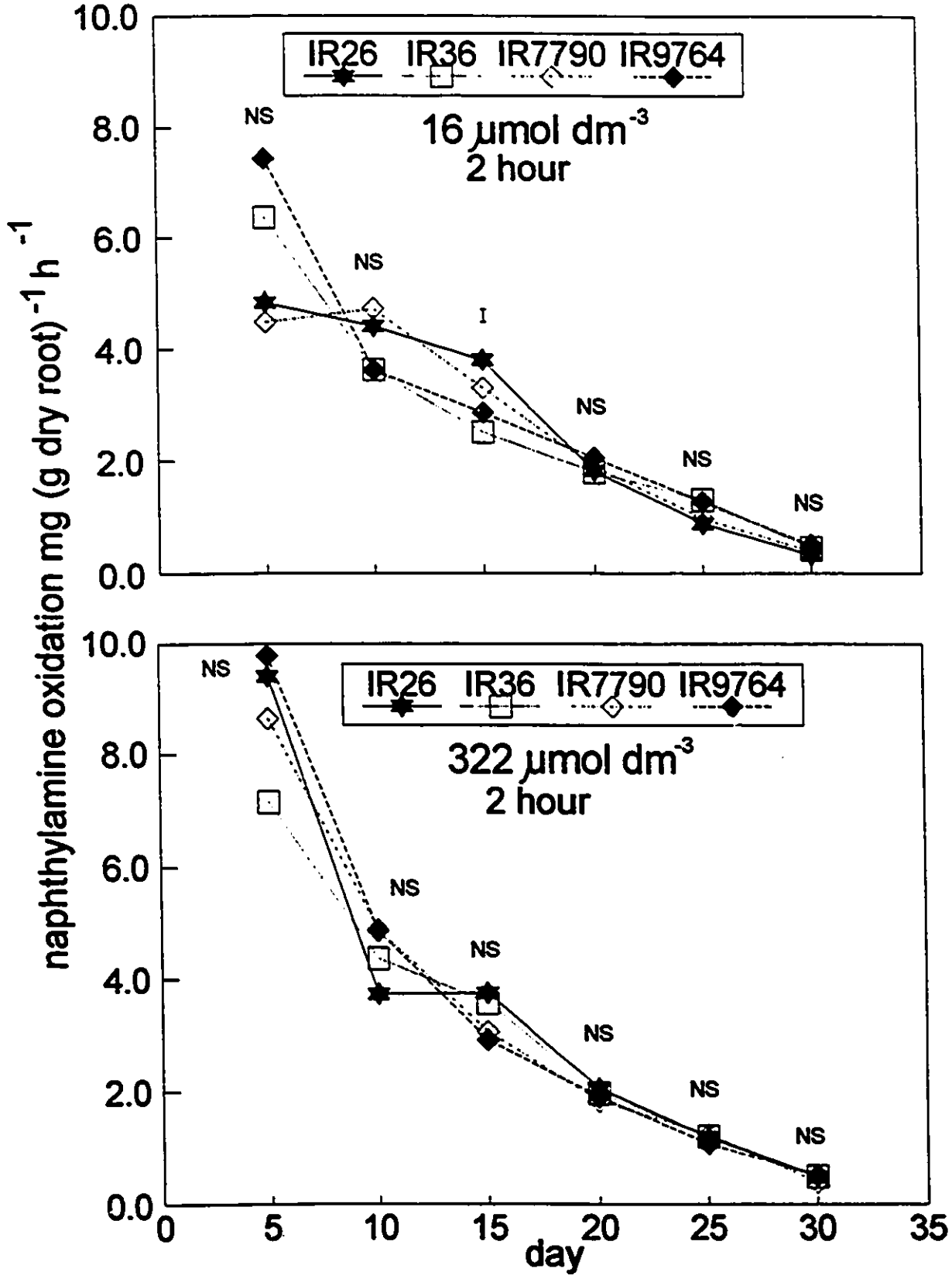
IR26 IR36 IR7790 IR9764
16 µmol dm-3
2 hour
322 µmol dm-3
2 hour
NS
I
naphthylamine oxidation mg (g dry root)-1 h-1
day
10.0
8.0
6.0
4.0
2.0
0.0
0
5
10
15
20
25
30
35

Fig ure 6. The 24-hour α-naphthylamine oxidation rate in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of four values.

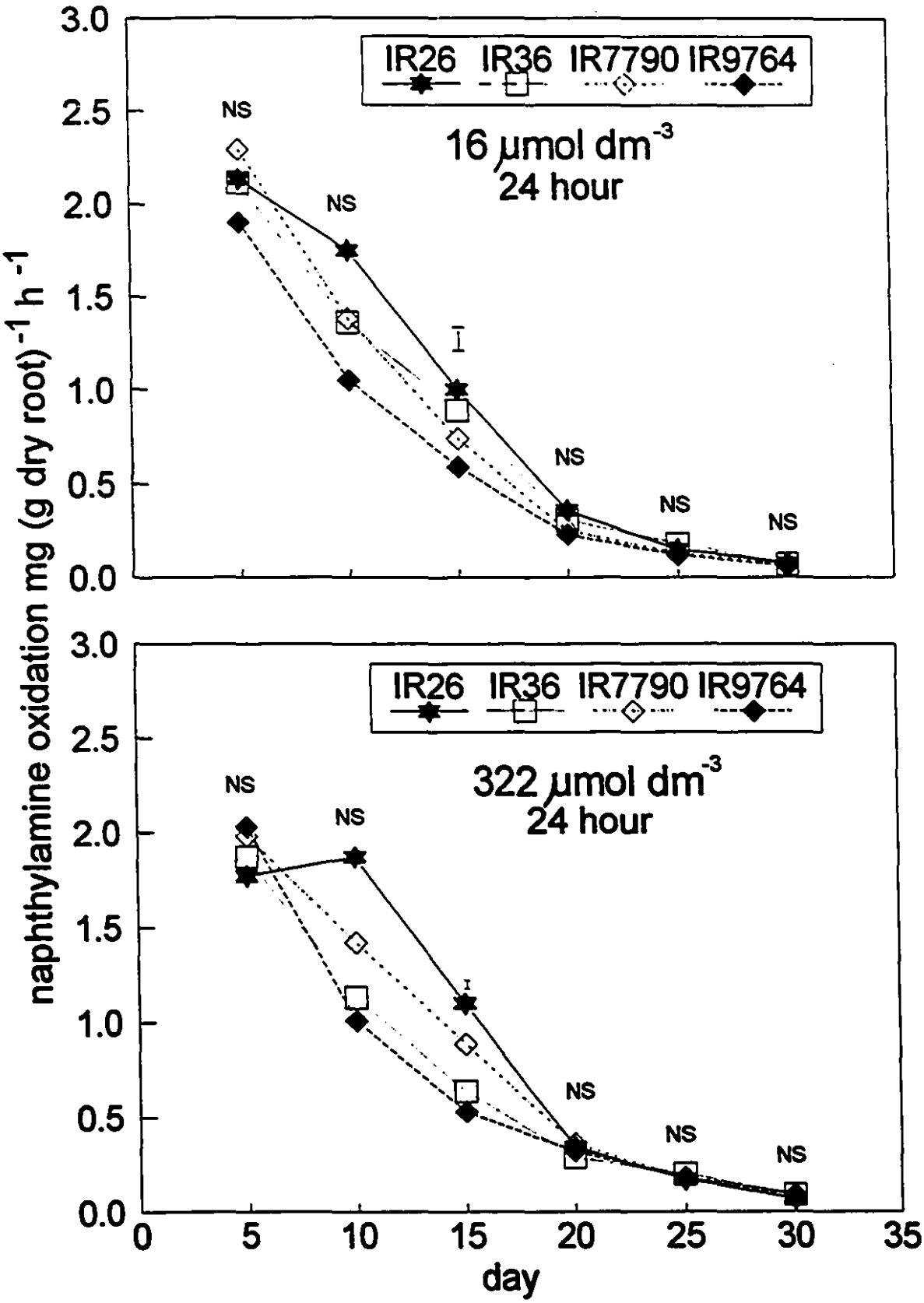
IR26 IR36 IR7790 IR9764
16 µmol dm-3
24 hour
322 µmol dm-3
24 hour
NS
naphthylamine oxidation mg (g dry root)-1 h-1
day
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
5
10
15
20
25
30
35

Figure 7. Measurement of pH changes along intact rice roots in 16 and 322 μmol P dm^{-3} solutions. Vertical bars represent LSD at $P < 0.05$ level. NS: means are not significantly different. Each point is a mean of two values.

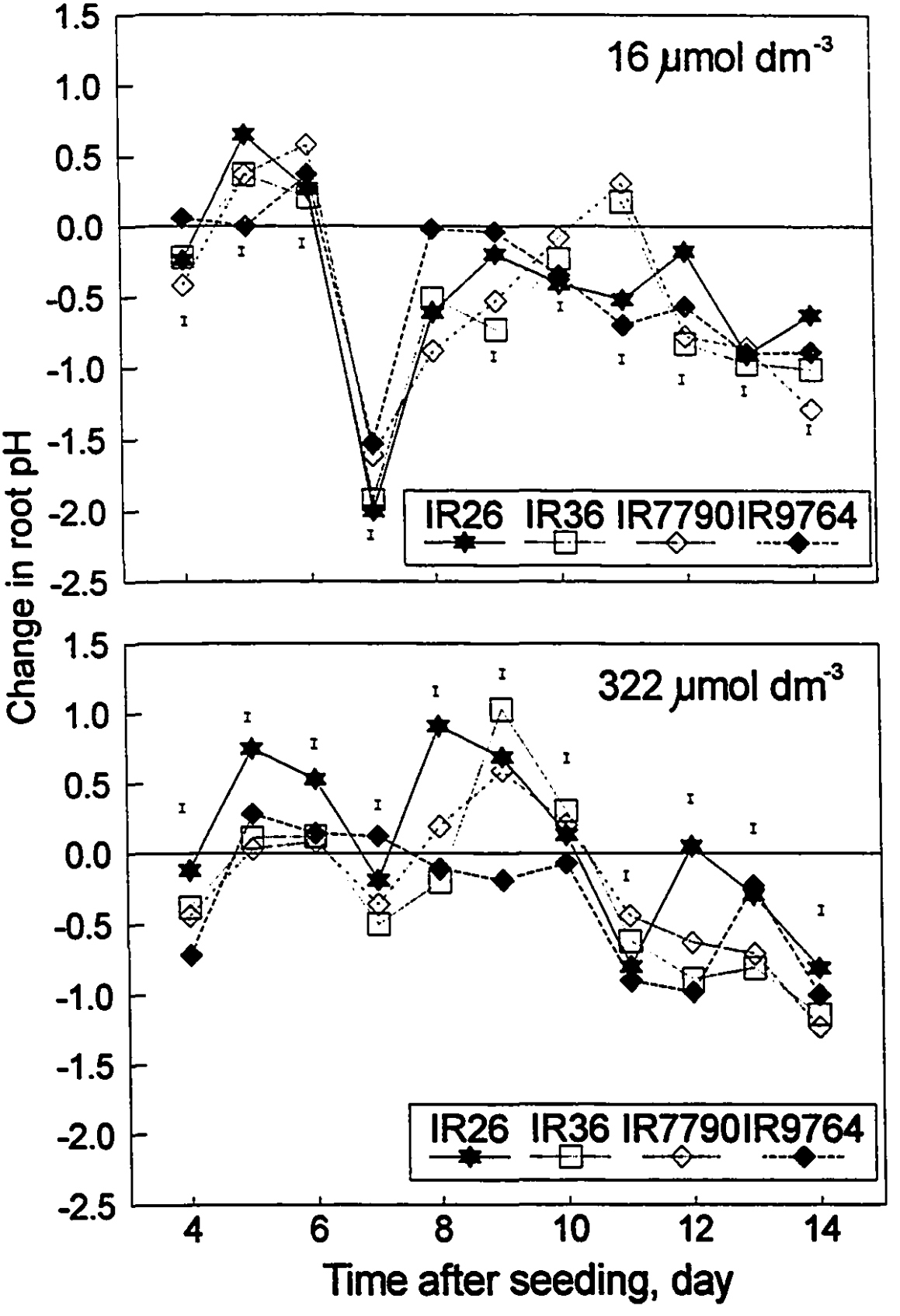
16 µmol dm-3
322 µmol dm-3
IR26 IR36 IR7790IR9764
Change in root pH
Time after seeding, day
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
4
6
8
10
12
14

Table 1. Analysis of variance for root and shoot length, root dry matter, and shoot: root ratio of rice and related to cultivar and P level of nutrient solution

Source of Variation	df	5 d	10 d	15 d	20 d	25 d	30 d
Root Length							
Phosphorus	1	*	NS	NS	*	NS	NS
Cultivar	3	NS	**	**	**	**	**
P * Cultivar	3	NS	NS	**	*	NS	*
CV, %		21	18	10	7	9	13
Shoot Length							
Phosphorus	1	NS	**	**	**	**	**
Cultivar	3	**	**	**	**	**	**
P * Cultivar	3	NS	NS	**	*	NS	NS
CV, %		8	9	6	5	7	10
Root DM							
Phosphorus	1	*	NS	NS	**	**	**
Cultivar	3	*	**	**	NS	NS	NS
P * Cultivar	3	NS	NS	NS	*	NS	NS
CV, %		26	24	26	20	21	29
Shoot/Root							
Phosphorus	1	**	*	**	**	**	**
Cultivar	3	NS	**	**	**	*	**
P * Cultivar	3	NS	NS	NS	*	NS	*
CV, %		20	20	10	10	11	16

*, ** significant at the $P < 0.05$ and $P < 0.01$ level by F test, respectively.

NS indicates not significant.

Table 2. Analysis of variance for α-naphthylamine oxidation rate of roots as related to cultivar, P level and sample time

Source of Variation	df	5 d	10 d	15 d	20 d	25 d	30 d
Cultivar	3	NS	**	**	*	**	**
Phosphorus	1	**	*	NS	NS	NS	NS
Sample Time	2	**	**	**	**	**	**
Cultivar * P	3	NS	NS	**	**	**	*
Cultivar * time	6	NS	*	NS	NS	NS	NS
P * time	2	**	NS	NS	NS	*	NS
Cultivar * P * time	6	NS	NS	NS	NS	*	NS
CV, %		35	18	22	16	15	24

*, ** significant at the $P < 0.05$ and $P < 0.01$ level by F test, respectively.

NS indicates not significant.

Table 3. Root length of four cultivars at two P levels after 15, 20 and 30 days of growth

Cultivar	P level	15 d	20 d	30 d
	μmol dm^{-3}	cm		
IR26	16	13.2b	19.5bc	26.0b
IR36	16	13.8b	20.6bc	21.9bc
IR7790	16	13.1b	20.8bc	28.9a
IR9764	16	20.0a	24.7a	30.6a
IR26	322	13.3b	18.8c	20.2c
IR36	322	12.8b	16.3d	28.2a
IR7790	322	18.1a	21.6b	28.4a
IR9764	322	17.8a	24.3a	29.8a

Mean of four replications.

Means in the same column with the same letters are not significantly different at the $P < 0.05$ level based on Duncan's Multiple Range Test.

CONNECTING PARAGRAPH

In Chapter II, the oxidizing activity and the root extrusion of H^+ from four rice cultivars were examined in relation to two levels of P. At the low P level, rice cultivars released increased amounts of H^+ due to an imbalance in cation-anion uptake. Differences among cultivars in root oxidizing activity, which is correlated with respiration (O_2 consumption), were affected by the P levels. A decrease in O_2 release to the rice rhizosphere (in anaerobic soils) could alter solid-solution chemistry of nutrients and potentially toxic elements. Low pH levels surrounding rice roots could modify nutrient uptake and cause the dissolution of some soil nutrients, such as P.

Oxidation of the rice rhizosphere is important as it maintains certain toxic elements such as Fe^{2+} in a precipitated form. However oxidation of Fe^{2+} will increase the acidification of the rhizosphere, further altering nutrient availability patterns. In the following Chapter, the effects of Fe^{2+} oxidation and pH changes in the rice rhizosphere are studied on a soil with a moderate Fe content, typical of many rice producing areas.

CHAPTER III

ROOT-INDUCED IRON OXIDATION AND pH CHANGES IN THE LOWLAND RICE RHIZOSPHERE

1. ABSTRACT

Measurements of profiles of ferrous and ferric iron and pH in blocks of reduced soil in contact with planar layers of rice (*Oryza sativa* L.) roots are reported. Initially 11-d-old plants were kept in contact with the soil for up to 12 d. Over periods of root-soil contact of up to 12 d, substantial quantities of iron were transferred towards the root plane producing a well-defined zone of ferric hydroxide accumulation. The pH in this zone fell by more than two units. The profiles changed with time. The decrease in pH was in part due to protons generated in ferrous iron oxidation, and in part due to protons released from the roots to balance excess intake of cations over anions, N being taken up chiefly as NH_4^+. But the decrease in pH was less than expected from the net acid production in these two processes, possibly because of proton consumption in CO_2 uptake by the roots. Because of the pH-dependence of soil acidity diffusion, the two sources of acidity greatly reinforce each other. Some implications for nutrient and toxin dynamics are discussed.

2. INTRODUCTION

It is well established that the ability of rice and other wetland plants to grow in flooded, anaerobic soils is due to their ability to transport oxygen to respiring root tissues via internal gas-channels (Drew, 1990; Armstrong et al., 1991). Less well understood are the extent to which this root-borne oxygen leaks out into the surrounding soil, and the extent of other root-induced changes that result from growth under anaerobic soil conditions. This paper reports measurements of root-induced changes in the rice rhizosphere and their causes.

In most flooded soils, the main reaction of oxygen is with mobile ferrous iron producing insoluble ferric hydroxide and acidity (Ahmad and Nye, 1990):

$$4Fe^{2+} + O_2 + 10H_2O \longrightarrow 4Fe(OH)_3 + 8H^+ \qquad (1)$$

Two additional processes may cause changes in acidity. One is the release of protons by roots to balance excess intake of cations over anions, plant-available N being chiefly in the form of NH_4^+ in anaerobic soil. The other is the transfer of CO_2 between the roots and soil: high CO_2 partial pressures arise in flooded soils, as well as within the roots, and therefore CO_2 may either be exported from the roots or taken up from the soil and transported to the atmosphere via the internal root gas channels (Higuchi, 1982; Higuchi et al., 1984). The pH changes caused by these processes and the accumulation of insoluble ferric hydroxide will together strongly influence processes in the rhizosphere. Some effects may be beneficial --

e.g., the suppression of toxic products of anaerobic metabolism such as Fe^{2+}, and the solubilization of sparingly soluble nutrients such as P and Zn (Otte et al., 1989; Jianguo and Shuman, 1991; Zhang et al., 1991); others may be detrimental – e.g., reduced mobility of NH_4^+ ions (Kirk and Bouldin, 1991; see below). We here report measurements of profiles of reduced and oxidized iron and pH changes near rice roots, and interpret them in terms of the above processes.

3. MATERIALS AND METHODS

1). The root-plane technique

To measure root-induced changes in the soil, we used an adaptation of the root-plane technique of Farr et al. (1969). In outline, plants were grown in thin nylon mesh bags in nutrient solution so that the roots formed a continuous planar layer. Each root plane was then sandwiched between cylinders of thoroughly reduced soil connected to water reservoirs, and the system sealed so that the only means of gas transfer between the atmosphere and soil was through the roots (Figure 1). After periods of plant growth, the cylinders were separated and the soil sectioned at narrow intervals parallel to the root plane to make analyses. The details follow.

2). Preparation of reduced soil cylinders

The experimental soil – an Epiaquult, properties in Table 1 – was obtained from Bo Capinang, San Dionisio, Iloilo, Philippines. The soil was chosen for its moderate Fe content and because it is non-swelling which was important for obtaining uniformly-packed soil cylinders. The < 0.2 mm fraction was used so that

the soil cylinders could be sectioned at 0.2 mm intervals to obtain reactant concentration profiles.

To obtain uniformly packed soil cylinders, it was found necessary to dry-pack pre-reduced soil and then re-flood it. Thereby the large volume of gas formed in soil reduction could be vented before packing the cylinders as follows. The sieved soil was puddled in 2 dm^3 1:2 (w/v) slurries with 5 mM $CaCl_2$ and allowed to thoroughly reduce for 3 to 4 weeks at 25°C with a daily 1 h purge with N_2 gas. After 3 to 4 weeks, the pH and redox potential (Eh) stabilized at approximately 6.5 and -150 mV, respectively. The soil was then dried to approximately 5% moisture by weight under O_2-free conditions. This was done by (i) placing the slurry in an anaerobic chamber, (ii) pouring off the supernatant solution and filtering the residue under suction whilst maintaining a positive pressure in the chamber with N_2 gas, and then (iii) spreading the filtered soil in a thin layer on the chamber floor and blowing dry N_2 gas (scrubbed to < 0.3% O_2 by volume) across it. The chamber atmosphere was kept dry with regularly changed silica-gel moisture traps (1 kg kg^{-1} dry soil). In this way approximately 2 kg of dry soil could be prepared in 6 h from two batches of slurry. Without opening the chamber, the soil was then packed into experimental cells and re-flooded with part of the anaerobic supernatant solution. The cells were 75 mm internal diameter Perspex tubing in 29 mm lengths. For packing, a Perspex plate containing a 10 mm diameter hole (for the water reservoir connection) covered with glass-fibre filter paper was fitted to one end of the cell, and dry soil packed

in to a bulk density of 1.16 kg dm^{-3}. The cylinders were then slowly re-flooded from below and allowed to re-equilibrate for 24 h. The final volumetric water contents were about 0.62:1 (v/v). Six pairs of cylinders were prepared for the one main experiment.

3). Preparation and growth of plants

Rice seedlings, cultivar IR9764, were grown in nutrient solution (Yoshida et al., 1976) for 11 d, by which time root lengths reached approximately 10 cm and shoot heights 24 to 30 cm, with 2 to 3 leaves per plant. Four plants were removed from the solution and their roots contained in circular, 75 mm diameter, 1 mm thick, 30 μm pore-diameter nylon mesh bags. (No root material should penetrate this mesh because rice does not form root hairs under flooded soil conditions and the finest roots are coarser than 30 μm). The roots were carefully arranged so as to form a planar layer.

Each "root plane" was then sandwiched between two of the reduced soil cylinders held under water to prevent oxidation. The cylinders were then sealed together with silicon sealant (Dow Corning aquarium silicon rubber) and the area around the stems filled with petroleum jelly to prevent gas exchange. Each cylinder was attached to a water reservoir so as to maintain a constant moisture tension in the cylinders during plant growth. The entire assembly was then placed in a controlled-environment growth chamber at 30/27°C day/night temperatures, 70% relative humidity, and 12 h photoperiod at 300 mol m^{-2} s^{-1} white light. Plants were grown for 3, 5, 8, 10 and 12 d. At harvest, the cylinders and plants were separated for analysis. A control was established in which solution-filled mesh

bags without plants were sealed between soil cylinders, and sampled after 12 d.

4). Soil analyses

The soil cylinders were quickly sectioned at approximately 0.2 mm intervals parallel to the root plane using a hand-microtome in a stainless-steel chassis that also held the cell in place, and a heavy stainless-steel blade. The sections were immediately immersed in 5 cm^3 of de-oxygenated 5 mM $CaCl_2$ (for later pH determination) to prevent oxidation. The sectioning and immersion took less than 20 s per section and preliminary experiments confirmed that very little oxidation took place. The section weights were determined from the total weights of the tubes plus $CaCl_2$ plus soil. Each section contained approximately 0.8 g of soil solid. The section thicknesses were calculated from their weights and the mean bulk density of the soil cylinder.

Soil pH was determined by shaking the sections end-over-end for 30 min at 25°C in 5 cm^3 of de-oxygenated 5 mM $CaCl_2$. Exchangeable plus solution Fe^{2+} was then determined by adding 10 cm^3 of 1 M NH_4OAc at pH 2.8 to the suspension and shaking end-over-end for 30 min at 25°C; and then Fe^{3+} was determined by shaking the centrifuged residue in 10 cm^3 of 2 M H_2SO_4, end-over-end for 30 min at 25°C. Fe^{2+} and Fe_{total} were measured in the centrifuged, filtered extracts using the 1,10-phenanthroline method; for Fe_{total}, Fe^{3+} was reduced with hydroxylamine hydrochloride (Olson and Ellis, 1982). Fe^{3+} was found by difference. With this method, the increase in Fe^{3+} measured upon complete re-oxidation of reduced slurry by bubbling with O_2 for 4 h matched exchangeable

plus solution Fe^{2+} measured in the slurry before oxidation.

5). Plant analyses

Oven-dry plant shoot and root materials were digested by the tri-acid method (Yoshida et al., 1976) and analyzed for Mg by atomic absorption spectroscopy, K and Ca by emission spectroscopy, and P by colorimetry. Total N was determined on separate plant samples by micro-Kjeldahl digestion. The Cl and S contents of the experimental material were not determined because the sample sizes were small. Instead, they were estimated from Cl and S contents of plants grown in the same soil under the conditions of the experiment. The values were 1.1 and 0.6 % by weight, respectively. The sums of the equivalents of cations (NH_4^+, K^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, $H_2PO_4^-$, SO_4^{2-}) taken up by the plants were calculated by assuming that all the N was absorbed as NH_4^+ and S as SO_4^{2-}. Note that Si, the other major mineral constituent of rice plants, enters the root as the uncharged H_4SiO_4 molecule.

6). pH buffer power of the soil during oxidation

Reduced soil slurry (250 cm^3) prepared as above were bubbled vigorously with air containing 5% CO_2 (roughly corresponding to the dissolved CO_2 content of the slurry) in a water-saturated atmosphere at 25°C for 36 h. The pH was monitored and at intervals, 2 cm^3 samples of slurry were withdrawn by pipette and analyzed for solution plus exchangeable Fe^{2+} using the method described above. Following Fe^{2+} extraction, the samples were oven-dried and the solution:soil ratios of the original 2 cm^3 samples determined. Concentrations of

solution plus exchangeable Fe^{2+} per unit weight of dry soil were calculated.

The soil pH buffer power - the change in concentration of titratable acidity required to produce unit change in pH - was found by plotting the calculated change in soil acidity at a given time against the pH measured at that time. The changes in acidity were calculated by assuming 2 mol of protons were produced for each mol of Fe^{2+} oxidized [Equation (1)]. From the concentrations of exchangeable NH_4^+ and Mn^{2+} in the reduced soil (Table 1), the quantity of H^+ produced in the oxidation of NH_4^+ and Mn^{2+} would have been negligible.

4. RESULTS AND DISCUSSION

1). Growth and morphology

The plants grew healthily after an initial "transplanting shock" lasting 1 or 2 d. Plant dry weights and nutrient absorption steadily increased over the growth period (Table 2). The cultivar used (IR 9764-45) is known for vigorous early growth. The lengths of nodal roots reached 20 cm in the 12 d experiments curling around within the bag. No roots penetrated the bag. The root surfaces appeared reddish-brown - indicating ferric hydroxide accumulation - except at the tips which remained white.

2). Changes in Fe^{2+} and Fe^{3+} in the soil

Profiles of Fe^{2+}, Fe^{3+} and pH after various periods of root-soil contact are shown in Figure 2. The concentration of Fe^{2+} in the bulk soil increased somewhat up to 5 d, suggesting that there was continuing soil reduction, presumably involving $Fe(OH)_3$ formed by reoxidation of Fe^{2+} when the soil was dried prior to

packing the cylinders. But after 5 d, the bulk Fe^{2+} concentration and pH stabilized and were similar to the values in the reduced soil slurry, indicating steady-state reduction and that there was no leakage of O_2 through the cylinder walls.

Figure 2 shows that a large quantity of Fe was transferred towards the root plane and oxidized, producing a well-defined zone of ferric hydroxide accumulation. Soil within this zone was strongly acidified. The zone of Fe^{2+} depletion was broader than that of Fe^{3+} accumulation, but of a similar width to the zone of strong acidification. The total quantities of Fe^{2+} oxidized and Fe^{3+} produced, calculated by summing the changes in amounts in each soil section relative to the bulk soil, were roughly equal (Table 3) indicating that our analytical methods were sound. In the experiments in which soil cylinders were incubated without plants, little oxidation took place (Figure 3), indicating that the seals were good and that in the planted systems the bulk of the O_2 entered the soil via the roots.

The Fe^{2+} concentration in our reduced experimental soil - approximately 80 mmol kg^{-1} - is not exceptional. Typically a fertile lowland rice soil contains 1 or 2 % dithionite extractable iron, and between 5 and 50 % of this is reduced to ferrous forms following soil flooding (Ponnamperuma, 1972). Thus, typical exchangeable Fe^{2+} contents are 10 to 200 mmol kg^{-1}.

3). Oxygen fluxes out of the roots

Table 4 gives the fluxes of O_2 out of the roots, averaged over the times between samplings, calculated from the increases in total quantities of Fe^{3+} per

cylinder by assuming that (i) 4 mol of Fe^{2+} reacted with each mol of O_2 [Equation (1)], (ii) the effective root surface area is the cross-sectional area in the root plane (0.44 dm^2), and (iii) there is no O_2 consumption in the rhizosphere in processes other than Fe^{2+} oxidation. The second assumption results in an overestimation because the true root surface area is rather larger than the plane being the sum of the curved surface areas of the roots facing the cylinder. But the planar geometry would tend to reduce the flux compared to that across an equal surface area of fine roots; note that a planar surface is equivalent to a cylindrical one of infinite radius, and thus an analogous comparison is that between one thick root and several finer roots of the same total surface area. The third assumption results in an underestimation of the O_2 flux because microbial O_2 consumption in the rhizosphere may be substantial. Howeler and Bouldin (1971) and Kirk and Solivas (1994) found that up to 50% of O_2 consumption by thoroughly reduced soils averaged over a few days was microbial. But this will vary greatly between soils depending on such factors as Fe content and organic matter content.

Values of O_2 loss reported by others vary from 10 nmol dm^{-2} (root surface) s^{-1} near root tips measured with cylindrical polarographic electrodes (Armstrong et al., 1991), to less than a hundredth of this for O_2 appearing in continuously replenished, O_2-free solutions bathing whole root systems (Bedford et al., 1991). The range is in part due to differences in leakiness between different parts of the root system, and in part to differences in the external O_2 sink. In soil, the O_2 sink is enhanced by diffusion of Fe^{2+} towards the root and its reaction with O_2.

The decline with time in the calculated rate of O_2 loss (Table 4) may be explained by (i) an increase in microbial O_2 consumption as the aerobic microbial population in the rhizosphere develops, and (ii) a decrease in the rate of oxidation of Fe^{2+} as the pH falls. This occurs because acidification decreases the fraction of exchangeable Fe^{2+} in the soil solid and, since the rate of oxidation of Fe^{2+} in the soil solid is much faster than that in solution, the net rate of oxidation is decreased (Ahmad and Nye, 1990; Kirk and Solivas, 1994). As the rate of Fe^{2+} oxidation declines, Fe^{2+} continues to diffuse in from the bulk soil and the Fe^{2+} profile becomes shallower (Figure 2e). Re-reduction of precipitated Fe^{3+} is evidently slow because there is little decrease in the Fe^{3+} concentration at the oxidation front as the O_2 flux declines.

Our experimental system averages the flux over all roots and over the whole lengths of roots. In practice, the flux will vary over the primary root length tending to be greatest in the region behind the root tip where the root is most O_2-leaky (Armstrong et al., 1991; Kumazawa, 1984). Generally, the root tip region appears white and marked ferric hydroxide coatings are only evident on older parts of the root. This is because, for a greater O_2 flux out of the root, Fe^{2+} diffuses a shorter distance toward the root before being oxidized. Hence the zone of Fe^{3+} precipitation near the root tip is more diffuse. Furthermore, the root tip is not in contact with the same portion of soil for very long, unless the root has stopped growing. In our experiments we did not find dramatic changes in soil colour in the zone of Fe^{3+} accumulation, indicating that rhizosphere 'oxidation' is not

necessarily apparent to the naked eye. In some circumstances, the peak of Fe^{3+} accumulation is not at the oxidizing surface but a little distance into the soil (Kirk et al., 1990).

In addition, there are likely to be differences between primary and lateral roots. By the end of the vegetative growth stage in rice, short fine lateral roots generally account for the bulk of root length (Drenth et al., 1991; Inada, 1967). The lateral roots may also contain cortical intercellular gas spaces (Butterbach-Bahl, 1993) through which O_2 diffuses from the primary roots' gas spaces, and having high surface area to volume ratios they tend to be O_2-leaky. In addition, O_2 may leak from the break in the primary root epidermis where the lateral root emerges. Consequently intense oxidation is observed along lateral root surfaces (Flessa and Fischer, 1992; Kawata and Ishihara, 1964) and O_2 leakage from laterals is calculated to be a substantial proportion of the total leakage to the soil (Armstrong et al., 1990). Since laterals account for the bulk of the total root length, they probably account for the bulk of nutrient intake and therefore nutrient intake must largely occur through zones of oxidation.

Enzymatic oxidation of Fe^{2+} is observed within and on the surfaces of rice roots (Ando et al., 1983; Kumazawa, 1984), but is unlikely to be quantitatively important in the rhizosphere because if the oxidizing agent responsible is synthesised in the root, its production is necessarily subordinate to the supply of molecular O_2 through the root.

4). Changes in soil pH

The changes in soil acidity corresponding to the measured pH profiles are shown in Table 5. These were calculated using the pH buffer power of the reduced soil suspension undergoing oxidation (Figure 4). The various possible causes of the pH changes are as follow.

(1) Fe^{2+} oxidation. Table 5 shows the quantities of acid produced in Fe^{2+} oxidation calculated from the quantity of Fe^{3+} formed using the stoichiometry of Equation (1). Acidification by this process is initially rapid, but declines as the O_2 efflux from the root declines.

(2) Cation-anion intake balance. The intakes of cations and anions from the soil at different times were calculated from the data in Table 2 with the quantities in the plants when placed in contact with the soil estimated by interpolation. The latter were 4.25 and 1.0 mmol per cylinder, respectively. The release of H^+ from the roots was then calculated from the intake of cations minus the intake of anions (Table 5). In making this calculation, we assume that all the N was absorbed as NH_4^+, but note that if any N was absorbed as NO_3^- due to nitrification of NH_4^+ in the rhizosphere, the net acid-base change would be the same because, although the roots export two protons less per NO_3^- absorbed in place of a NH_4^+, two protons are formed in the nitrification of each NH_4^+.

At 3 d, the release of H^+ to balance cation-anion intake is small compared with the H^+ generated in Fe^{2+} oxidation, but at later times the two are of similar magnitude. In the early stages, the sum of the two roughly agrees with the

calculated total acidification. But after 8 d, the sum exceeds the total acidification.

(3) CO_2 uptake or release. The roots may either release CO_2 generated in their respiration or, because very high concentrations of dissolved CO_2 arise in flooded soils (equivalent CO_2 partial pressure = 0.5 to 20 kPa (Ponnamperuma, 1972), the roots may passively absorb CO_2 and convey it to the atmosphere via their internal gas channels. Net CO_2 uptake would remove acidity from the soil ($HCO_3^- + H^+ \longrightarrow CO_2 + H_2O$) and would explain the discrepancy between the observed net acidification and the sum of acidity produced in Fe^{2+} oxidation and in cation-anion intake imbalance. The discrepancy is approximately 4 mmol per cylinder after 12 d (Table 5). The CO_2 production required to balance CO_2 loss equivalent to this acidity consumption is approximately 2 mmol kg^{-1} d^{-1}, which is well within likely rates of CO_2 production in continuing soil reduction in the soil cylinder. Note that the soil pH buffer power was determined under conditions of constant, high CO_2 partial pressure, so acid consumption in CO_2 loss did not occur.

(4) Shape of the pH profile. This is governed by the rate of propagation of the pH change away from the roots into the soil bulk. The factors governing this rate are summarized in the soil acidity diffusion coefficient, D_{HS}, which for most practical purposes is given by (Nye, 1981)

$$D_{HS} = \frac{2.303\ \vartheta\ f}{b_{HS}} (D_{LH}\ [H_3O^+] + D_{LC}\ [HCO_3^-]) \qquad (2)$$

where b_{HS} = soil pH buffer power, ϑ = soil water content, f = diffusion impedance

factor, and D_{LH} and D_{LC} = diffusion coefficients of H_3O^+ and HCO_3^- in free solution. The explanation of this equation is that pH changes are propagated through soil by the movements of conjugate acid-base pairs: acids from regions of low pH to high and bases in the opposite direction. The first term in the bracket accounts for the acid-base pair H_3O^+ - H_2O; the second term for the pair H_2CO_3 - HCO_3^-. D_{HS} is therefore strongly pH-dependent and passes through a minimum in the pH range 4.5 to 5.5 where $[H_3O^+]$ and $[HCO_3^-]$ are both low. In this pH range, therefore, a flux of acid or base through the soil will cause steep pH gradients, as shown in Figure 2. A consequence is that the effects of proton release from the roots and proton release in Fe^{2+} oxidation are supplementary, and the effect of the two acting together is greater than their effects in isolation.

Equation (2) also shows that D_{HS} will be high in flooded soils because θf and the CO_2 partial pressure are both high. The high value and pH-dependence of D_{HS} explain why the pH drop near the root plane changes very abruptly with time (Figure 2). After 8 d, the O_2 flux out of the roots and the consequent rate of acidification due to Fe^{2+} oxidation are low. Thus D_{HS} increases, and, although proton release from the roots is increased, the resulting pH change is spread further into the soil and the pH change at the root surface is less.

5. IMPLICATIONS

We have found a very substantial transfer of Fe towards the root surface and a large fall in the soil pH due to the combined effects of protons generated in Fe^{2+} oxidation and protons released from the roots. The fall in pH may have

been moderated by uptake of CO_2 from the soil. We should expect similar Fe accumulations and pH changes in soils with similar Fe contents, even though their aerobic pHs may be above neutral (Kirk et al., 1990).

The extent to which O_2 leakage from roots is in the plant's best interest is unclear. Any leakage is of course at the expense of the O_2 supply to respiring root tissues. Some leakage is necessary to lessen the concentrations of Fe^{2+} and other toxic products of anaerobic microbial metabolism in the soil. Also, acidification may help solubilize less available soil nutrients such as P. Such solubilization may be crucial because, once a soil has reached steady state following flooding, a large part of the P is in immobile but acid-soluble forms (for example, associated with ferrous carbonates and hydroxides or with negatively-charged surfaces to which Fe^{2+} acts as a bridging ion; Kirk et al., 1990), and it is necessary for the plant to solubilize this P before it can be taken up. But a cost to the plant will be impaired access of NH_4^+ ions to root surfaces (Kirk and Bouldin, 1991; Kirk and Solivas, 1994). This occurs because the major anion balancing cations in flooded soil solutions is HCO_3^- (in aerobic soils it is generally NO_3^-) and the concentration of HCO_3^- falls as the pH falls so that the concentration of cations maintained in solution also falls. This is the dominant effect of acidification on NH_4^+ mobility although, NH_4^+ mobility will be modified by the decrease in exchangeable Fe^{2+} with oxidation, tending to increase cation sorption, and the effect of acidity on soil surface charge, tending to decrease cation sorption. These factors are important because the rate of NH_4^+ uptake is in most circumstances diffusion-limited, even

in water-saturated soil and even without acidification-induced immobilization. Acidification may also impede the mineralization of soil N in the rhizosphere and N fixation by rhizosphere microbes.

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Figure 1. Exploded view of the experimental system.

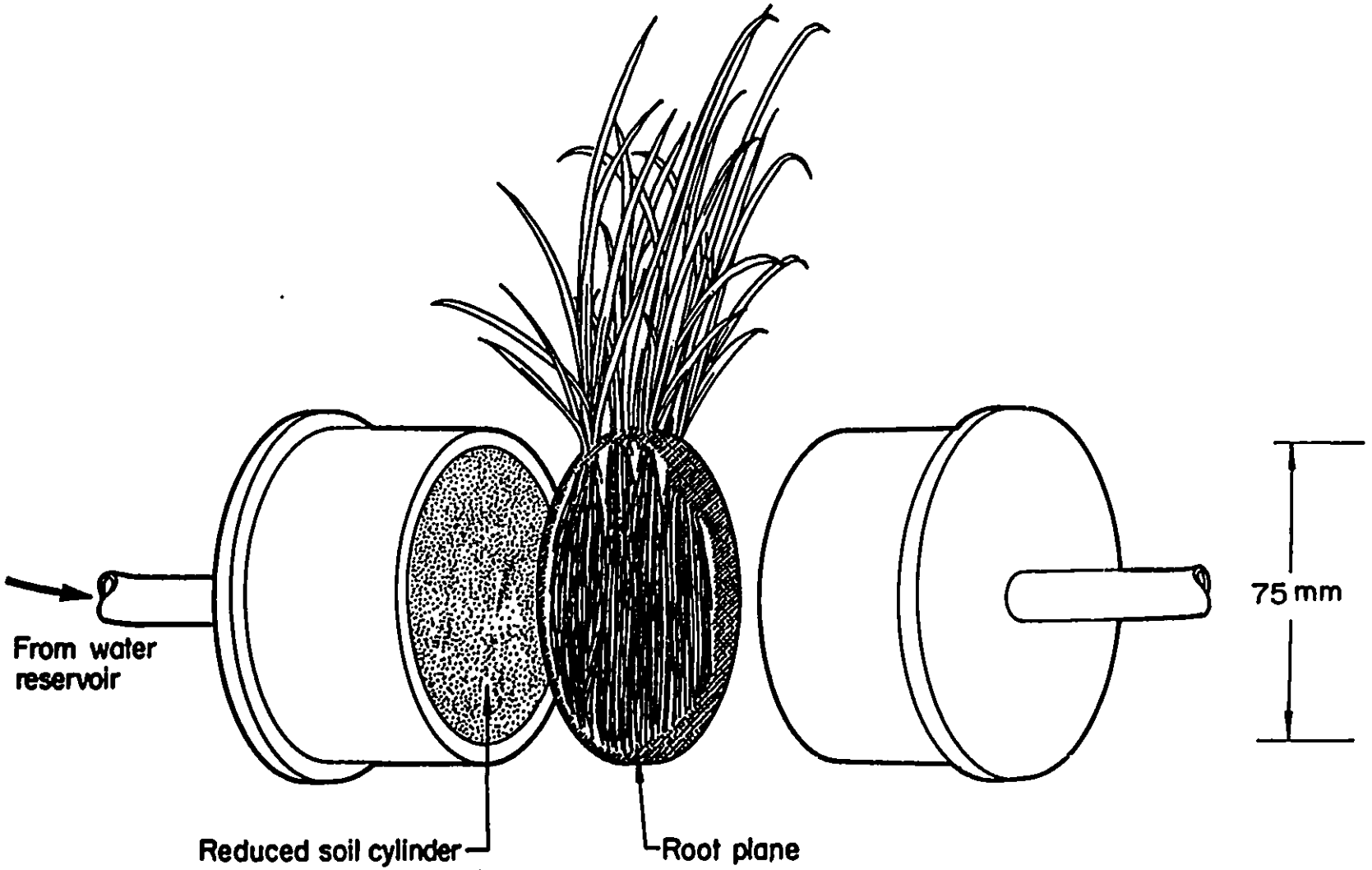
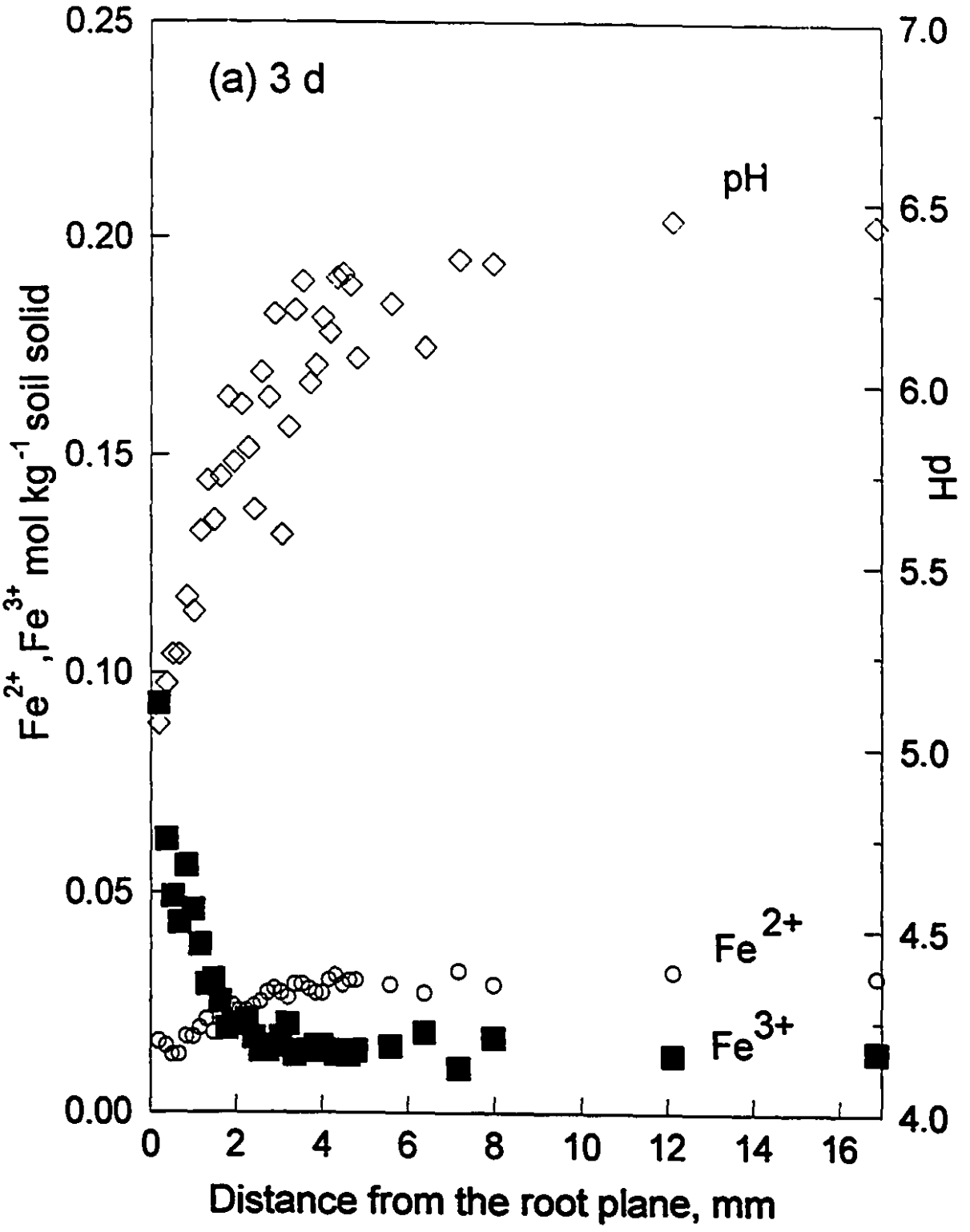
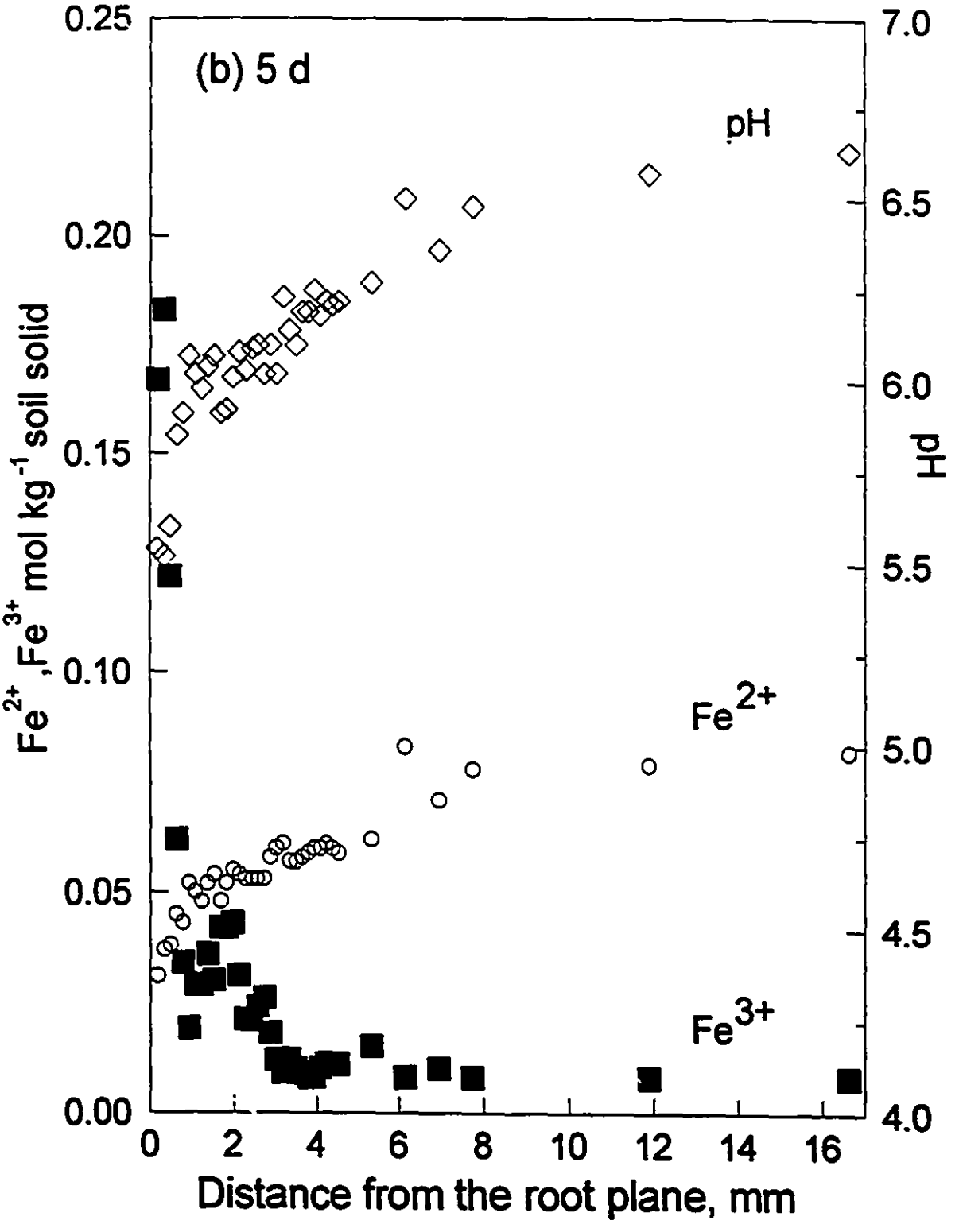
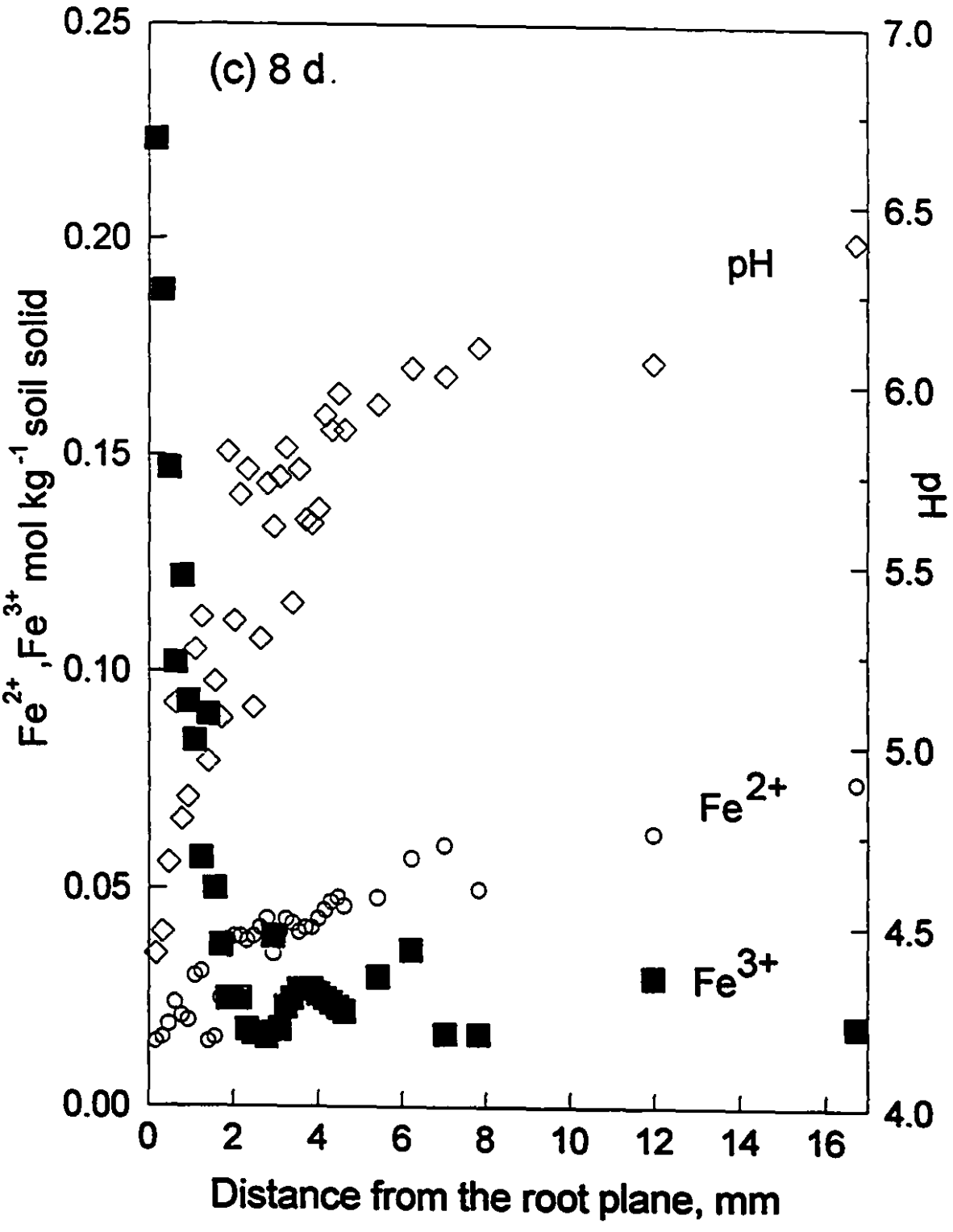
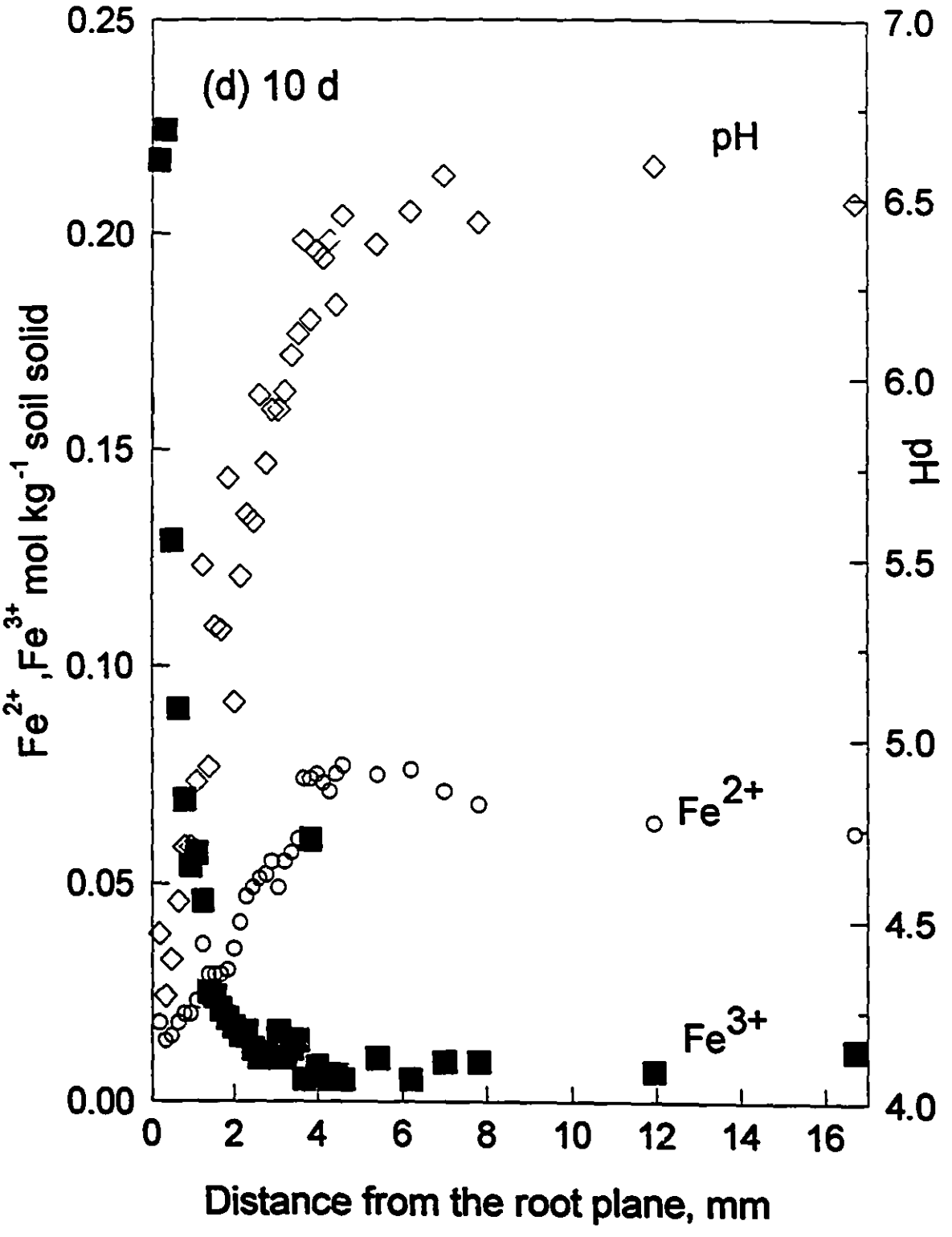

From water reservoir
Reduced soil cylinder
Root plane
75 mm

Figure 2. Concentration profiles in the region of the root plane after periods of root-soil contact. Graphs 2a, 2b, 2c, 2d and 2e represent sample periods 3, 5, 8, 10 and 12 d, respectively.


(a) 3 d
pH
Fe^{2+}
Fe^{3+}
Fe^{2+},Fe^{3+} mol kg^{-1} soil solid
pH
Distance from the root plane, mm
0.25
0.20
0.15
0.10
0.05
0.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
2
4
6
8
10
12
14
16


(b) 5 d
pH
Fe^{2+}
Fe^{3+}
Fe^{2+},Fe^{3+} mol kg^{-1} soil solid
pH
Distance from the root plane, mm
0.25
0.20
0.15
0.10
0.05
0.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
2
4
6
8
10
12
14
16


(c) 8 d.
pH
Fe^{2+}
Fe^{3+}
Fe^{2+},Fe^{3+} mol kg^{-1} soil solid
pH
Distance from the root plane, mm
0.25
0.20
0.15
0.10
0.05
0.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
2
4
6
8
10
12
14
16


(d) 10 d
pH
Fe^{2+}
Fe^{3+}
Fe^{2+}, Fe^{3+} mol kg^{-1} soil solid
pH
Distance from the root plane, mm
0.25
0.20
0.15
0.10
0.05
0.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
2
4
6
8
10
12
14
16

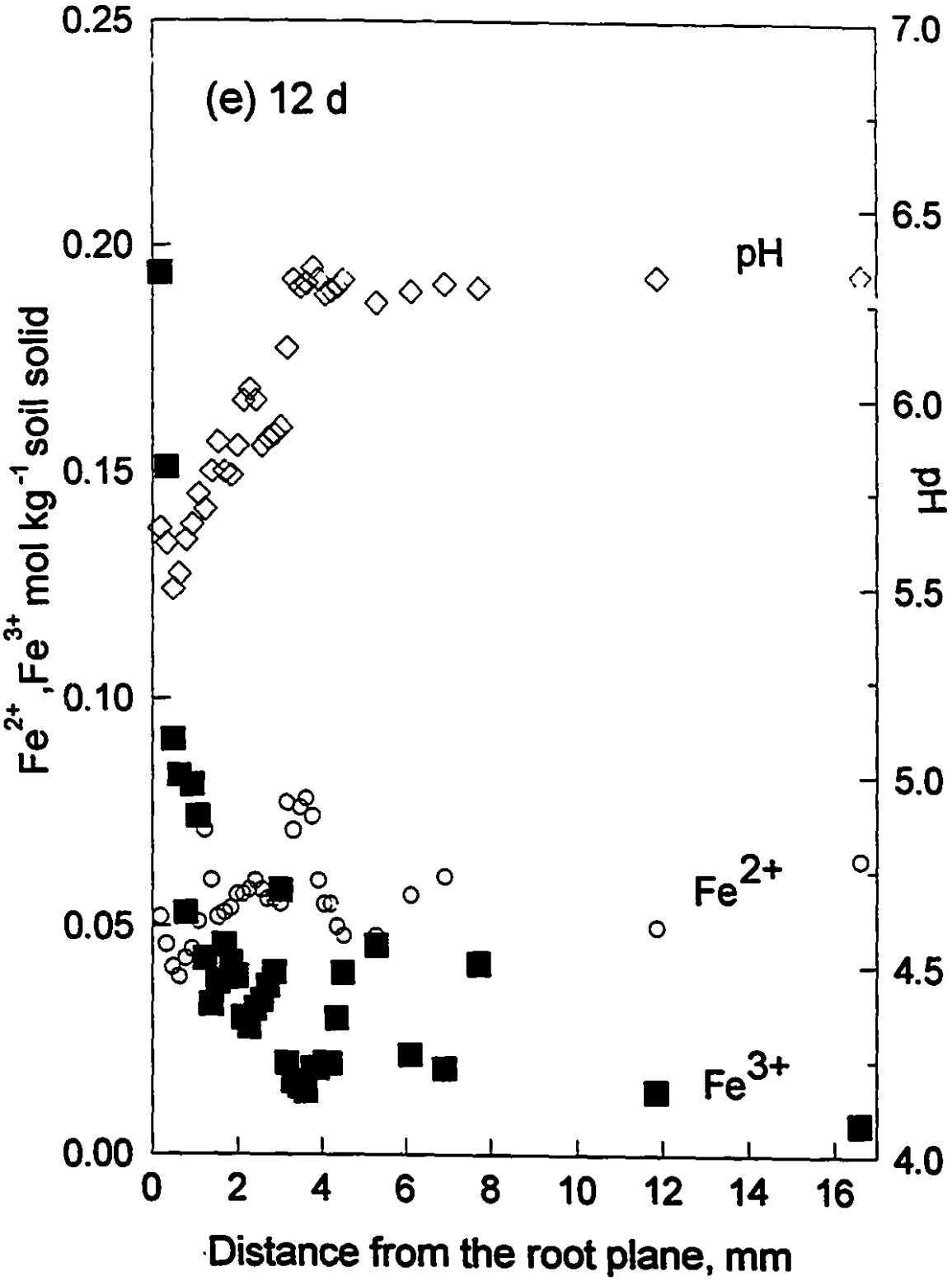

(e) 12 d
pH
Fe^{2+}
Fe^{3+}
Fe^{2+},Fe^{3+} mol kg^{-1} soil solid
pH
Distance from the root plane, mm
0.25
0.20
0.15
0.10
0.05
0.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
2
4
6
8
10
12
14
16

Figure 3. Concentration profiles in the experimental system without plants after 12 d.

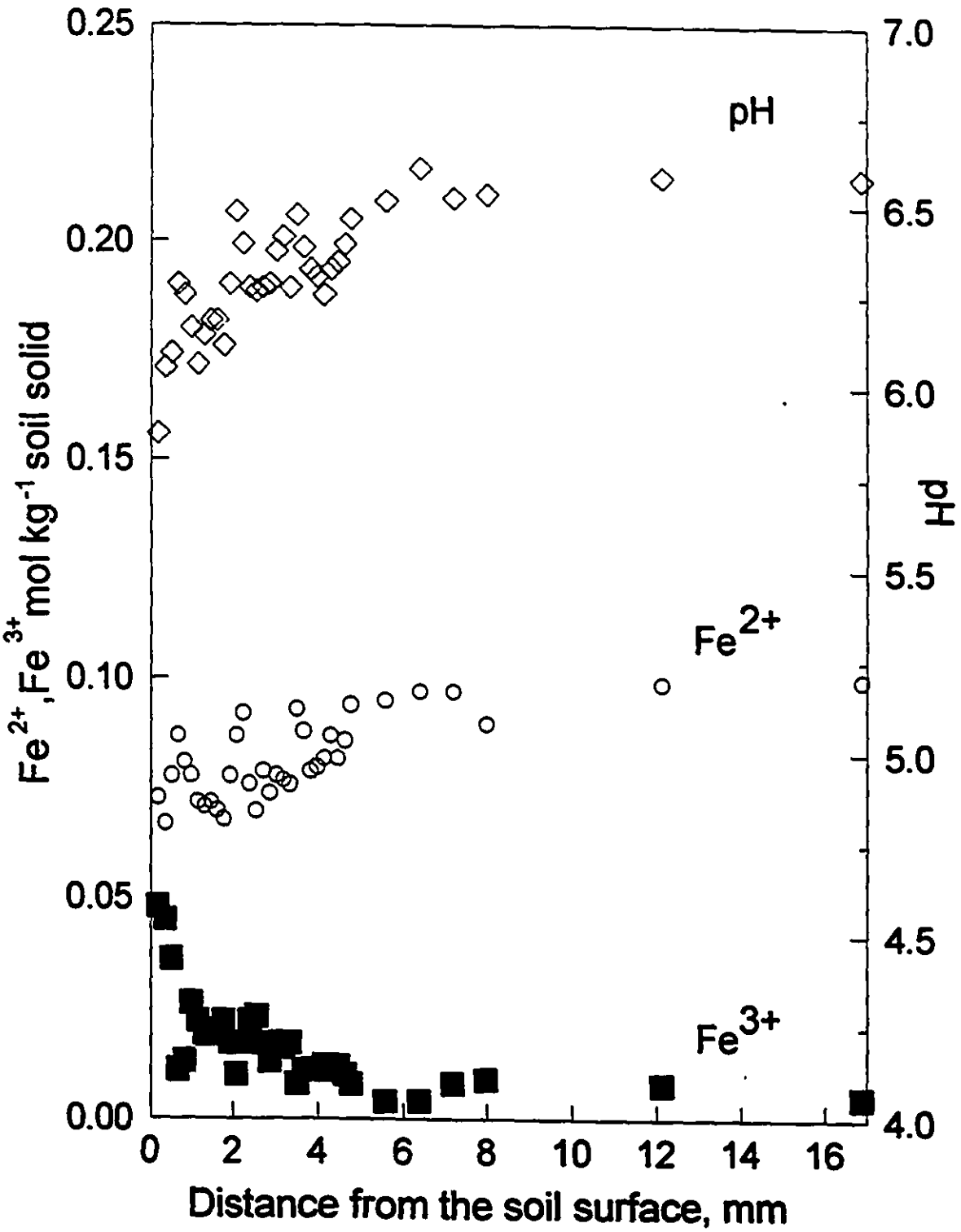

0.25
0.20
0.15
0.10
0.05
0.00
7.0
6.5
6.0
5.5
5.0
4.5
4.0
pH
Fe2+
Fe3+
Fe2+,Fe3+ mol kg-1 soil solid
0
2
4
6
8
10
12
14
16
Distance from the soil surface, mm

Figure 4. The soil pH buffer curve inferred from changes in Fe^{2+} and pH during oxidation of reduced soil slurry by calculating acid production from Fe^{2+} consumption and plotting this against pH. The pH buffer power is the inverse of the slope = 0.093 mol (H^+) kg^{-1} (soil) pH^{-1} (R^2 = 0.99).

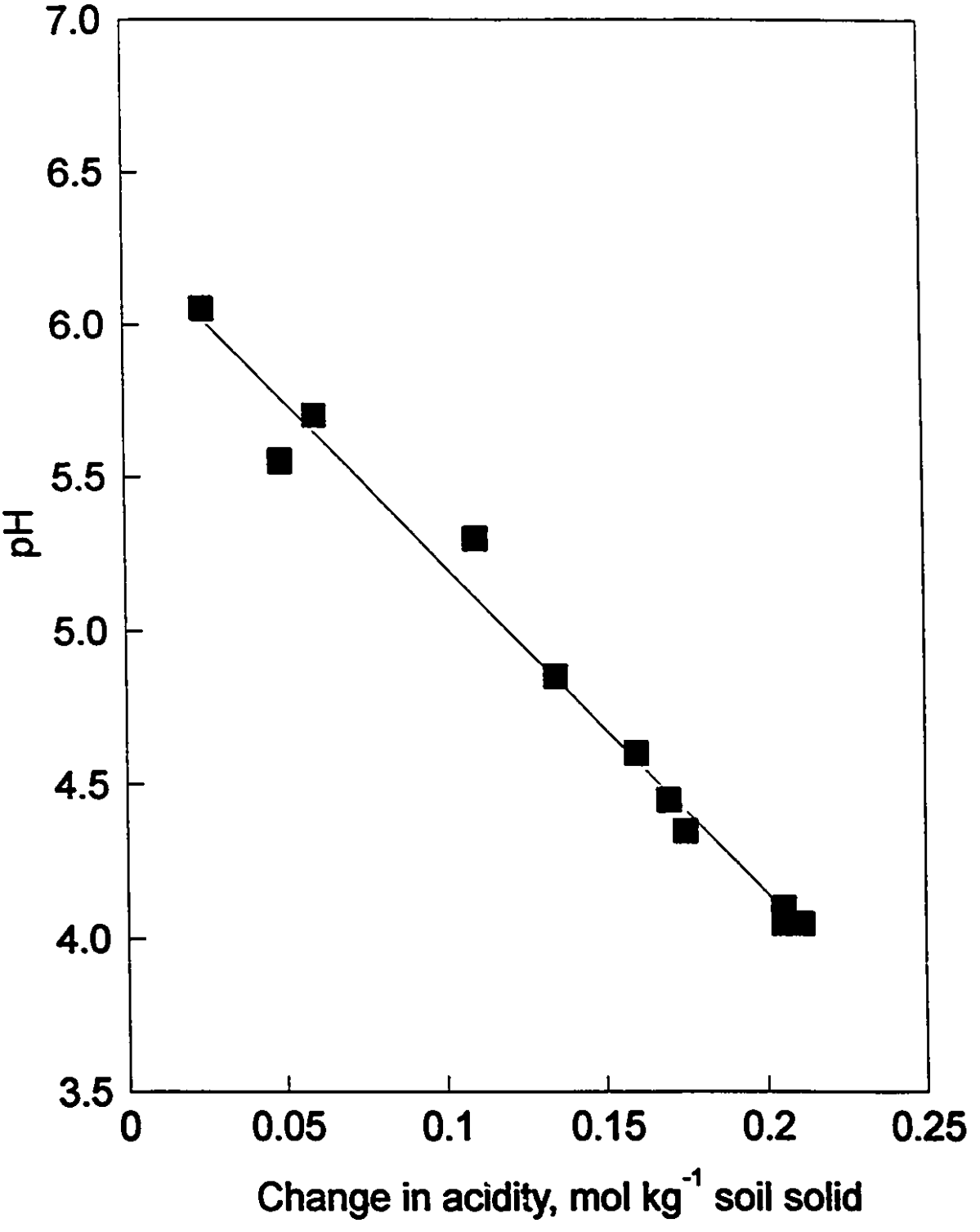

7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
pH
0
0.05
0.1
0.15
0.2
0.25
Change in acidity, mol kg-1 soil solid

Table 1. Properties of San Raphael loam

Property	value
Texture	Sandy loam
Aerobic pH†	3.4
Anaerobic pH‡	6.6
C, g kg^{-1}	12.1
N, g kg^{-1}	1.0
Extractable cations in reduced soils‡	
Ca^{2+} mmol kg^{-1}	16.6
Mg^{2+} mmol kg^{-1}	12.0
Fe^{2+} mmol kg^{-1}	174.6
Mn^{2+} mmol kg^{-1}	< 1
K^{+} mmol kg^{-1}	3.6
Na^{+} mmol kg^{-1}	5.8
NH_4^{+} mmol kg^{-1}	< 1

† 1 : 5 (H_2O).

‡ NH_4^{+} extracted in 1 M KCl, others in 1 M ammonium acetate.

Table 2. Net plant dry weights and mineral contents per experimental system at different sampling times

Sampling day	Plant dry wt.	Plant mineral contents				
		N	P	K	Ca	Mg
	g	% wt./wt.				
3	2.86	3.51	0.27	1.22	0.20	0.37
5	3.12	4.00	0.28	1.35	0.25	0.33
8	3.59	4.12	0.29	1.35	0.31	0.38
10	3.80	4.19	0.29	1.02	0.32	0.42
12	4.99	4.23	0.31	1.00	0.34	0.44

Table 3. Total quantities of Fe^{2+} consumed and Fe^{3+} formed in each cylinder at different sampling times

Sampling day	Fe^{2+} consumed	Fe^{3+} formed
	— mmol cylinder^{-1} ——	
3	0.33	0.55
5	0.98	0.87
8	1.74	1.65
10	1.89	1.79
12	1.95	1.82

Table 4. Calculated O_2 fluxes across the root-plane surface averaged over the times between samplings

Sampling interval	Mean O_2 flux
d	nmol dm^{-2} s^{-1}
0-3	1.20
3-5	1.05
5-8	1.71
8-10	0.46
10-12	0.10

Table 5. Calculated total quantities of H^+ formed and quantities of H^+ released in Fe^{2+} oxidation and released from the roots to balance excess uptake of cations over anions.

Sampling day	Total H^+ formed	H^+ formed in Fe oxidation	Cations taken up by plants	Anions taken up by plants	H^+ exported from roots
	mmol cylinder^{-1}				
3	2.17	1.10	0.37	0.12	0.25
5	2.25	1.74	1.37	0.22	1.15
8	3.93	3.30	2.49	0.40	2.09
10	3.46	3.58	2.88	0.50	2.38
12	3.66	3.64	5.26	0.96	4.30

CONNECTING PARAGRAPH

Chapter III showed that Fe^{2+} moved toward the rice root surface and a large decrease in rhizosphere pH resulted from the combined effects of H^+ produced from Fe^{2+} oxidation and H^+ released from the roots. Oxygen release from rice roots is passive and the extent to which this release is of benefit to the plants is unclear.

A large portion of P found in anaerobic soils is in immobile but acid-soluble forms. Acidification of the soil within the influence of the roots could affect P reactions, increase P solubilization and subsequent uptake of P by rice. In Chapter IV, the effect of the low rhizosphere pH on soil P fractions found in anaerobic soils is evaluated. This experiment was conducted on the same soil and with the same rice cultivar as previous experiments.

CHAPTER IV

CHANGES IN SOIL PHOSPHOROUS FRACTIONS IN THE RHIZOSPHERE OF LOWLAND RICE

1. ABSTRACT

Lowland rice (*Oryza Sativa*) may be limited in growth and yield by phosphorous (P) deficiency on certain soils in South East Asia. Rice roots release O_2, exudates and protons into the rhizosphere that could alter P chemistry in reduced soils. The objective was to determine the effect of rice roots on P fractions in a reduced Tropudult deficient in P. Profiles of P fractions and pH were obtained in reduced soil in contact with planar layers of rice roots for 12 d. Phosphorous fractions were: anion-exchange resin P; alkali -inorganic and -organic P in 0.1 M NaOH; acid soluble P in 1 M HCl + 1 M H_2SO_4; and residual P. Acid soluble P decreased in the soil block within four mm of the root plane. The zone of depletion coincided with a zone of acidification. Alkali -inorganic and -organic P varied with distance from the root plane and showed an accumulation near the roots. Lowland rice plants growing in flooded soil may benefit by the release of acid soluble P through root-induced acidification.

2. INTRODUCTION

Phosphorous deficiencies are increasing constraints as P is 'mined' from soils under intensive rice production, and as rice production is extended into marginal soils where P levels may be low (Neue and Zhonglin, 1990). Knowledge of the mechanisms by which rice thrives on these soils would be useful in management programs.

Rice root oxidation of anaerobic soil may alter P chemistry in the rhizosphere. Rice roots reduced soil pH and enhanced oxidation of ferrous Fe (Fe^{2+}) (Begg et al., 1994). The pH in the root-influenced zone was often one to two units lower than that in the bulk soil.

Larsen (1967, 1969), with the use of ^{32}P, observed that rice roots appeared to be absorbing P that was not in rapid equilibrium with the soil solution, that is, soil P was solubilized by reactions within the rhizosphere.

Processes causing increases in solution P following flooding included 1) reductive dissolution of solid phase P, 2) dissolution due to acid-base changes, and 3) dissolution by organic anions (Willett, 1986; Kirk et al., 1990). Processes subsequently causing decreases in solution P included 1) sorption on slowly formed solid phases, 2) slow sorption by existing solid phases, and 3) microbial P uptake and microbial degradation of organic anions occupying sorption sites.

Acidification near roots reported in Begg et al. (1994) caused by protons generated by both Fe^{2+} oxidation and excess intake of cations over anions could cause the dissolution of P in the rhizosphere. Concurrent with this process, freshly

precipitated, amorphous ferrous hydroxides and carbonates formed by the precipitation of Fe^{2+} at low pH could have a large P sorbing capacity. These materials have high specific surfaces and consequently high P sorption capacities. The sorbed P may become occluded as precipitation continues. The ability of rice to obtain sufficient P from the reduced soil would depend on the balance between increased P sorption on ferric hydroxides and increased release from acid-soluble forms. Objectives were to measure changes in P fractions in the rice rhizosphere and the dimensions of these changes as a consequence of acidification.

3. MATERIALS AND METHODS

1). Preparation of reduced soil cylinders

The experimental soil was obtained at a depth of 0 to 20 cm from a San Raphael loam, an epiaquic mixed isohyperthermic Tropudult (Table 1) obtained at Iloilo, Philippines. The soil was air-dried, ground and sieved < 0.2 mm. Sieved soil was puddled in 2 dm^3, 1:2 slurries with 5 mM $CaCl_2$ and reduced for four weeks at 25°C with a daily 1 h purge with N_2 gas. After four weeks, the pH and Eh stabilized at near 6.5 and -150 mV, respectively. Slurries were transferred to an anaerobic chamber, the supernatant was removed and the residue filtered under suction while maintaining a positive pressure in the chamber with N_2 gas. The filtered soil was spread in a thin layer and dried by circulating N_2 gas (< 0.3% O_2 by volume) across the soil. Moisture was removed from the chamber atmosphere by silica-gel traps (1 kg gel kg^{-1} dry soil).

Within the chamber, dry soil (approximately 5% moisture) was ground and

packed into experimental cylinders of 75 mm internal diameter Perspex tubing in 29 mm lengths. The closed end of the cylinder was a Perspex plate with a 10 mm diameter hole covered with glass-fibre filter paper. Cylinders were slowly re-saturated by capillary rise from beneath with the original anaerobic supernatant solution and allowed to re-equilibrate for 24 h.

Dry bulk density was 1.16 kg dm^{-3}. Final volumetric water contents averaged 0.62 dm^3 dm^{-3}. Six pairs of cylinders were prepared for each of two replicates. One pair of cylinders was prepared as a control.

2). Growth of plants and preparation of cells

Rice seedlings, cultivar IR9764, were grown for 11 days, in nutrient solution (Yoshida et al., 1976). Greenhouse temperatures ranged between 30°C at night and 45°C during the day. Relative humidity varied between 50% at night and 100% during the day. When the seedlings were removed from the solution root lengths had reached approximately 10 cm and shoot heights 24 to 30 cm with two to three leaves per plant.

Four plants were arranged to form a planar root layer in circular, 75 mm diameter, 1 mm thick, 30 μm pore-diameter nylon mesh bags. Each "root plane" was sandwiched between two of the reduced soil cylinders recently removed from the anaerobic chamber and held under water to prevent oxidation. Two cylinders were sealed with silicon sealant (aquarium silicon rubber, Dow Corning, Australia) and the area around the stems filled with petroleum jelly to prevent gas exchange. The control cylinders contained only a mesh bag. Each cylinder was

attached to a water reservoir to maintain a constant soil moisture content during plant growth. The assembly was placed in a controlled-environment growth chamber at 30°C/27°C day/night temperatures, 70% relative humidity, and 12 h photoperiod at 300 mol m^{-2} s^{-1}. Plants were grown for an additional 3, 5, 8, 10 and 12 d. The control was sampled after 12 d. At harvest, cylinders and plants were separated for analysis. Each replicate series ran at different dates, under identical experimental conditions.

Soil cylinders were sectioned at approximately 0.2 mm intervals parallel to the root plane using a hand-microtome. To prevent oxidation sections were immediately immersed in 5 cm^3 of de-oxygenated 5 mM $CaCl_2$ before pH determination and P fractionation. Moisture contents were determined for each cylinder to calculate soil section weights. Phosphorous analyses were carried out on one of the two cylinders in each run.

3). Soil P fractionation

Phosphorous fractionation followed a modification of the Hedley et al. (1982) procedure. All extractions were made under O_2-free conditions by displacing air from the extraction tubes (35 cm^3 centrifuge tubes) with N_2 and placing the tubes containing soil plus extractant in water-filled bottles during shaking. Resin beads (Dowex 1 x 8) contained in polyester mesh bags as described by Sibbesen (1978) were used for resin extraction. Washings from the resin were returned to the main suspension and water-soluble inorganic P was determined by centrifuging the suspension at 10,000 RPM for 600 s and filtering. Filtrants

from each subsequent extraction were centrifuged and filtered in the same manner.

Inorganic P (alkali-i) in the extracts and digests was determined after adjustment to between pH 5 and 6 by the Murphy and Riley (1962) method. Total P was determined on digests of 10 cm^3 aliquots of the NaOH extracts using the method of Thomas et al. (1967). Organic P (alkali-o) was determined as the difference between total P in the NaOH extraction digests and inorganic P in the NaOH extractions.

Fractionation estimated the following soil P pools.

1. Anion exchange resin: inorganic P considered plant-available (Amer et al., 1955).

2. 0.1 M NaOH (Alkali): some labile organic P (Saunder, 1956; Anderson, 1964), some Fe and Al phosphates (Saunder, 1956; Chang, 1976), and P associated with positively-charged oxide surfaces (White, 1980).

3. 1 M HCl + 1 M H_2SO_4 (Acid): Ca phosphates (Chang, 1976), P associated with amorphous ferrous hydroxides and carbonates (Ponnamperuma, 1985) and P associated with negatively-charged soil surfaces through exchangeable cations (White, 1980).

4. $H_2SO_{4\ conc.}$ - H_2O_2 digest (Residual): occluded phosphates and recalcitrant organic forms.

Total P was calculated as the sum of all the other P fractions for that sample interval.

Net changes in the P fractions per soil cylinder were obtained by summing differences in concentrations of each fraction across 0 to 8 mm from the root plane. Changes were found in each section by subtraction from the bulk soil concentrations multiplied by weight of the section. Bulk soil concentrations of each fraction were obtained from the means of sections 10 to 17 mm from the root plane.

4). Recovery of added P

Two anaerobic soil slurries were prepared as for the paired cylinders. To one slurry, 64 μmol P g^{-1} was added as KH_2PO_4 and the slurry plus P equilibrated for 2 d. Phosphorous fractions were determined on five samples from each slurry after 2 d.

5). Plant analysis

Oven-dry (80°C for 48 h) plant shoot and root materials were digested by the tri-acid method and analyzed for P colorimetrically (Yoshida et al., 1976). The quantity of P in the plants when placed in contact with the soil was estimated at 244 ± 20 μmol by extrapolation back to 11 d of growth using the equation $y = 244 - 4.46x + 1.34x^2$ ($R^2 = .99$). This equation was obtained using plant P uptake data, where y is the P uptake and x is time in days.

6). Statistics

Data were analyzed as a factorial arrangement with day and distance from the root plane as factors, in a randomized complete block design with two replications. T-tests were used to compare slurry and bulk P fractions. Regression

equations were calculated using means of replicates.

4. RESULTS

1). P fractions in control slurries

Distribution of P fractions in the reduced slurries indicated less than 0.02 $\pm$ 0.01 μmol g^{-1}, or 3% of total P, was water-soluble or resin-extractable (Table 2). This value is too low to provide for plant requirements (Yoshida, 1981). The rest of the extracted P was distributed as: alkali-i, 13%; alkali-o, 9%; acid 15%; and residual 60%. In the slurry with added P, all of the added P was recovered by the procedure. Twenty-two percent of the added P was found in the acid soluble P fraction. Alkali -inorganic and -organic fractions increased by 37% and 46%, respectively to 30% and 36% of total P.

2). Plant growth

The plants remained visibly healthy throughout the experiment after a temporary wilting due to the transfer from water culture to soil. After 12 d, there were no visible signs of plant P deficiency.

Although plant dry matter (root plus shoot) showed no significant difference among days based on an analysis of variance (Table 3), the regression curve of dry matter gain with time was significant ($P < 0.01$ level) (Figure 1).

Plant P content significantly increased during the 12 days ($R^2 = 0.99$) (Figure 2). Calculated absorbed P showed no significant difference among days (Table 3) but the curvilinear relationship between absorbed P and time was positive and significant ($P < 0.01$ level) (Figure 3).

3). Changes in profiles of soil P fractions with distance from the root plane

In the paired cylinders without plants, differences in pH were less than 0.5 pH units between the bulk soil and the soil adjacent to the nylon mesh bag. This showed that the seals were good and that in the planted systems the bulk of the O_2 entered the soil via the aerenchyma to the roots. If air entered the system from other than the roots the control would have shown oxidation of Fe^{2+} on the soil surface and a corresponding decrease in pH.

Mean bulk values (10 to 17 mm from the root plane) of all P fractions did not significantly change with distance nor time.

In the paired cylinders with plants there was no significant difference among days in water soluble and resin extractable P during the 12 d sample period. Values were less than 0.01 for water-P and 0.15 μmol P g^{-1} for resin-P.

The mean bulk alkali-i P concentration was 1.80 $\pm$ 0.23 μmol P g^{-1} which was significantly higher ($P < 0.01$ level) than 1.40 $\pm$ 0.10 μmol P g^{-1} in the control slurry. Levels in the 0 to 8 mm distances from the roots of alkali-i P were significantly different ($P < 0.05$ level) among days, but there was no discernible trend. Alkali-i P did not differ significantly with distance from the root plane (Figures 4b to 5b).

Alkali-o P in the control slurry was 0.38 $\pm$ 0.04 μmol P g^{-1}, which was significantly lower ($P < 0.01$ level) than 1.08 $\pm$ 0.25 μmol P g^{-1} extracted from the bulk soil. Levels in the 0 to 8 mm distances of alkali-o P differed significantly (P

< 0.01 lower) with distance and among days. Increases in alkali-o P were evident up to 4 mm from the root plane but did not display similar increases throughout the sample period (Figures 4b to 5b).

The mean bulk soil concentration of acid P in the cylinders was 2.02 ± 0.29 μmol P g^{-1} which was not significantly different from the 2.22 ± 0.09 extracted from the control slurry. Acid soluble P within 0 to 8 mm from the root plane ranged from 1.50 to 2.25 μmol P g^{-1}. Variability between replicates was high with a C.V. of 14%. Acid-P (0 to 8 mm) had significant differences ($P < 0.01$ level) between days and with distance from the root plane. There was a depletion of acid soluble P within 4 mm of the root plane and this effect increased with time (Figures 6a to 7a). The depletion zone corresponded to the zone of acidification (Figures 6b to 7b).

Residual P in the slurry was 13.6 ± 0.9 μmol P g^{-1} (Table 2) which was not significantly different from 12.4 ± 0.2 μmol P g^{-1} extracted from the bulk soil. Changes in residual P (0 to 8 mm) were significantly different ($P < 0.01$ level) during the sample period and with distance from the root plane (Figures 4a to 5a).

4). Mass balances of P in the soil and plants

Mean values of total P depletions, (Table 4), were less than mean values of calculated P absorbed by plants at day 3, 5 and 12 (Table 3), but values overlaid limits of the standard error, indicating that the soil depletion of P was equal to plant uptake of P. Changes in concentration of all P fractions were not significantly different among days (Figure 8). Regression equations of changes in

concentration of P fractions through the sample period were significant ($P < 0.05$ level) for the acid and residual P fractions and highly significant ($P < 0.01$ level) for the summed total change in P (Table 5).

5. DISCUSSION

Rice cultivar IR9764 is scored tolerant to low levels of P (Lantin, 1989, personal communication). Plant dry matter and calculated P absorbed by plant showed similar curvilinear relationships. The initial transplanting shock slowed the gain in dry matter through days three and five but subsequently plant dry matter showed a rapid increase. The calculated P-absorbed curve exhibited a similar relationship, suggesting that roots were actively absorbing P after 5 d.

Although each replication of the rhizosphere study was conducted in a plant growth chamber under identical environmental conditions, seedlings were grown in a greenhouse under ambient conditions at different times. Part of the variability in results may be due to differences in the initial growth among seedlings. The root plane was 90% complete within the nylon mesh bag upon the start of the experiment. New roots grew in a circular pattern and remained within the nylon bag for the duration of the experiment. Although O_2 fluxes differ along the root length according to age, it was impossible to differentiate between old and new roots in this experiment.

The P fractionation scheme as proposed for anaerobic soils recovered almost all of the added P in the soil slurry experiment. The scheme is suitable for determining P fractions in anaerobic soils during plant growth.

Lower values of residual P in soil cylinder experiments compared with the control slurry and the gradual increase of the residual P in the soil cylinders with time were probably the result of the drying and re-wetting procedure used to prepare the soil cylinders. Drying may have caused P adsorption on newly formed ferric oxides, and re-wetting would release some P into solution by hydrolysis.

The parallel depletion of acid soluble P and decrease in pH near the root plane supports the idea that solubilization of P occurred along with acidification of the rhizosphere. The acidification was due to the oxidation of Fe^{2+}, which diffused towards higher O_2 contents near the root surfaces of the rhizosphere and release of protons due to excess uptake of cations over anions by rice plants (Begg et al., 1994). Jianguo and Shuman (1991) reported a similar depletion of acid soluble P in rice rhizospheres.

Saleque and Kirk (1995) in a subsequent study on P fractions in the rice rhizosphere split the acid soluble P into two pools. The most P solubilized in the rhizosphere originated from acid soluble P extracted with an anion exchange resin and a H^+ -form cation exchange resin. Prediction of P solubilized with pH decreases near the rice root surface using soil buffer capacities for P and pH and other parameters corresponded values obtained in their experiments (Kirk and Saleque, 1995).

Both the alkali-i and alkali-o fractions tended to increase near the root plane and subsequently decreased after 4 mm from the root plane. The alkali-o P

increase may be caused by P immobilization by rhizosphere microbes stimulated by root-released C compounds (Yoshida, 1976). Large fluctuations in the alkali-o P values suggest a dynamic pool, which probably was in a state of flux depending on O_2 concentration and readily oxidizable C of the rhizosphere. The significant difference between alkali-o in the control slurry (0.38 μmol P g^{-1}) and mean alkali-o within 8 mm of the root plane (1.31 μmol P g^{-1}) supports the idea that some solubilized P was immobilized in microbial tissue in the cylinder study. Neither alkali-i nor alkali-o extractable P was depleted by plant uptake. This compares with Jianguo and Shuman (1991) who found little plant depletion of the alkali P fraction. Saleque and Kirk (1995) found 10% of the total P depletions resulted from solubilization of the alkali-i P fraction.

The increase in alkali-i P near the root plane may be due to P association with ferric hydroxide formed by Fe^{2+} oxidation or precipitation as Fe phosphates. The CO_2 concentration would be higher in the rhizosphere compared to the bulk soil due to root and microbial respiration, which may increase the quantity of P associated with amorphous ferrous hydroxides and carbonates (Ponnamperuma, 1985).

The mass balance calculation of total P depleted from the cylinders (mean = 33 μmol P per cylinder) compares with the calculated P absorbed by plant per cylinder (mean = 30 P μmol per cylinder). An error associated with the mass balance calculation was that calculated total P error was equal to the sum of errors on individual fractions.

Regression curves of changes (Δ P) in acid, residual and total P through 0 to 8 mm from the root plane displayed an initial rapid depletion of P. At day 10, depletion of P had slowed indicating that most of the available P had been taken up by the plant.

The methodology for P fractionation in anaerobic soils requires that sample preparation and P extraction be done under anaerobic conditions to prevent changes in P fractions on exposure to O_2 in the air. The organic P fraction showed varying concentrations with distance from the root plane (0 to 8 mm), no discernible trend between days and a large C.V. between replicates.

The results indicate that P was depleted from the acid P fraction, which was important to the nutrition of rice but the fractionation scheme was not suitable in determining if alkali-o P contributed to the total P uptake by the plant. The methodology was suitable for delineating inorganic sources of P that had lower variability compared to the organic fraction. Additionally, the methodology shows the depth of soil from the root plane altered by rice roots.

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Figure 1. Changes in mean values of plant dry matter with time.

**, significant at $P < 0.01$ level.

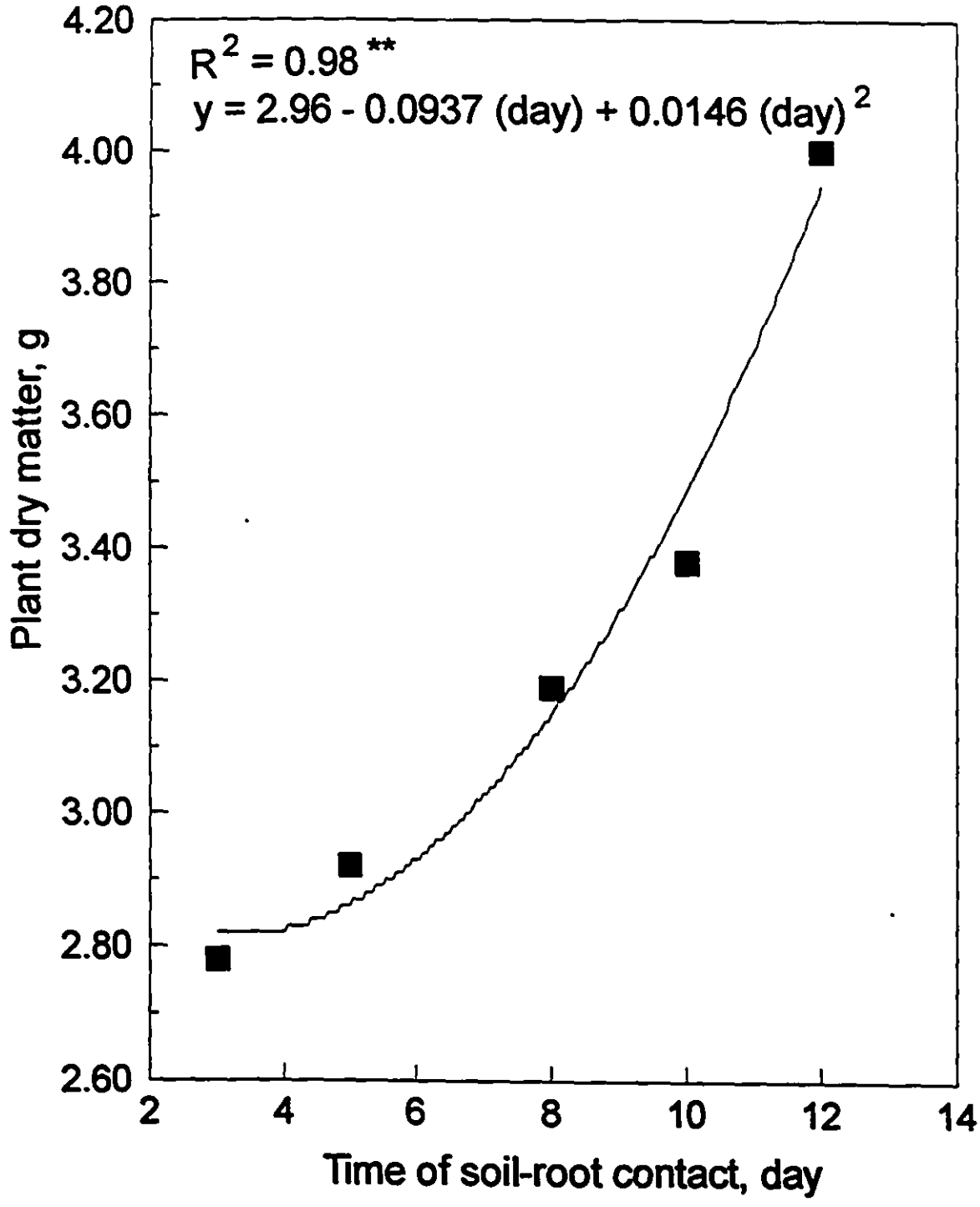

$R^2 = 0.98$ **
$y = 2.96 - 0.0937\ (day) + 0.0146\ (day)^2$
Plant dry matter, g
4.20
4.00
3.80
3.60
3.40
3.20
3.00
2.80
2.60
2
4
6
8
10
12
14
Time of soil-root contact, day

Figure 2. Changes in mean values of plant P content with time.

**, significant at $P < 0.01$ level.

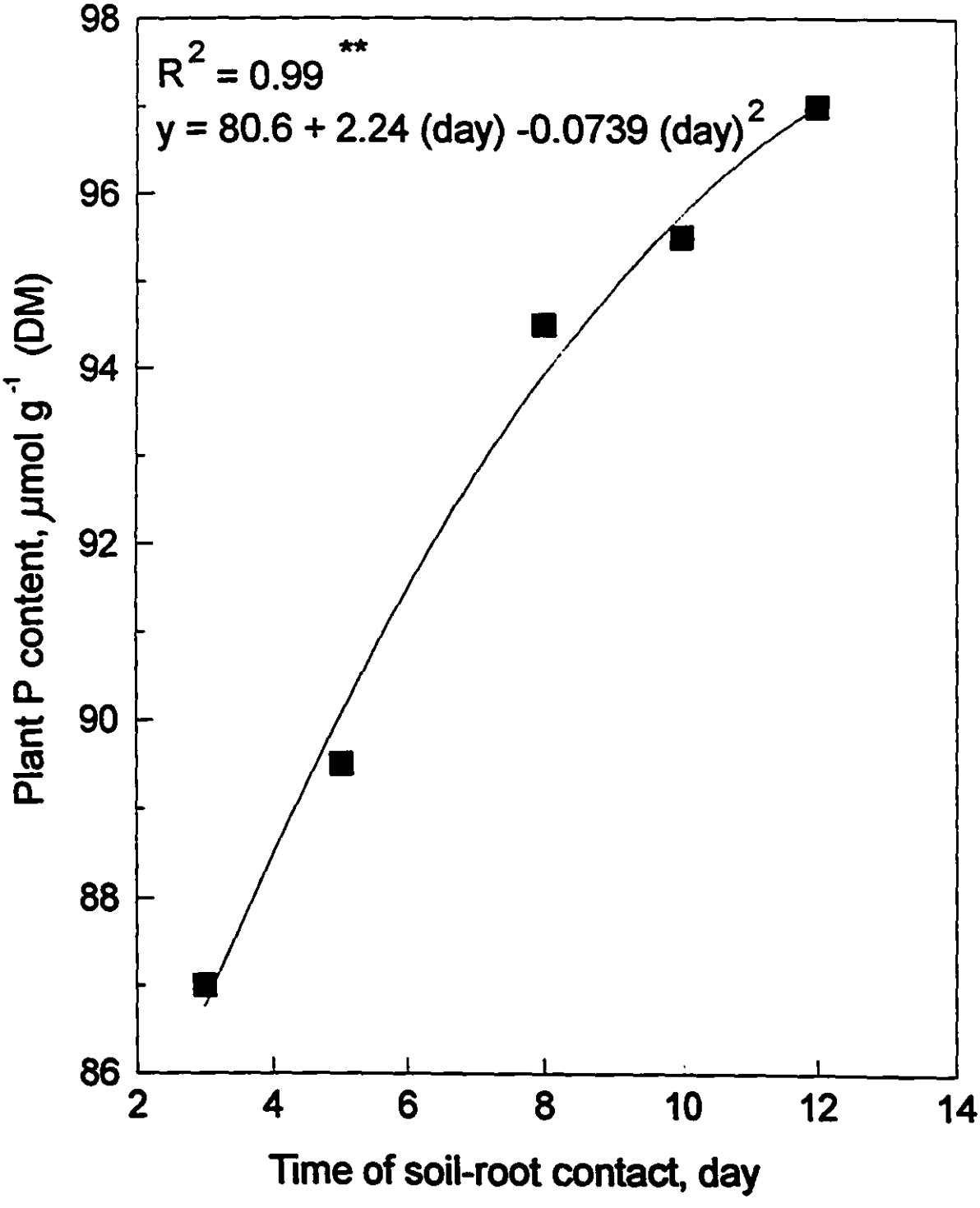

$R^2 = 0.99$ **
$y = 80.6 + 2.24\ (day) - 0.0739\ (day)^2$
Plant P content, μmol g^{-1} (DM)
Time of soil-root contact, day
98
96
94
92
90
88
86
2
4
6
8
10
12
14

Figure 3. Changes in mean values of calculated P absorbed by plant with time. **, significant at $P < 0.01$ level.

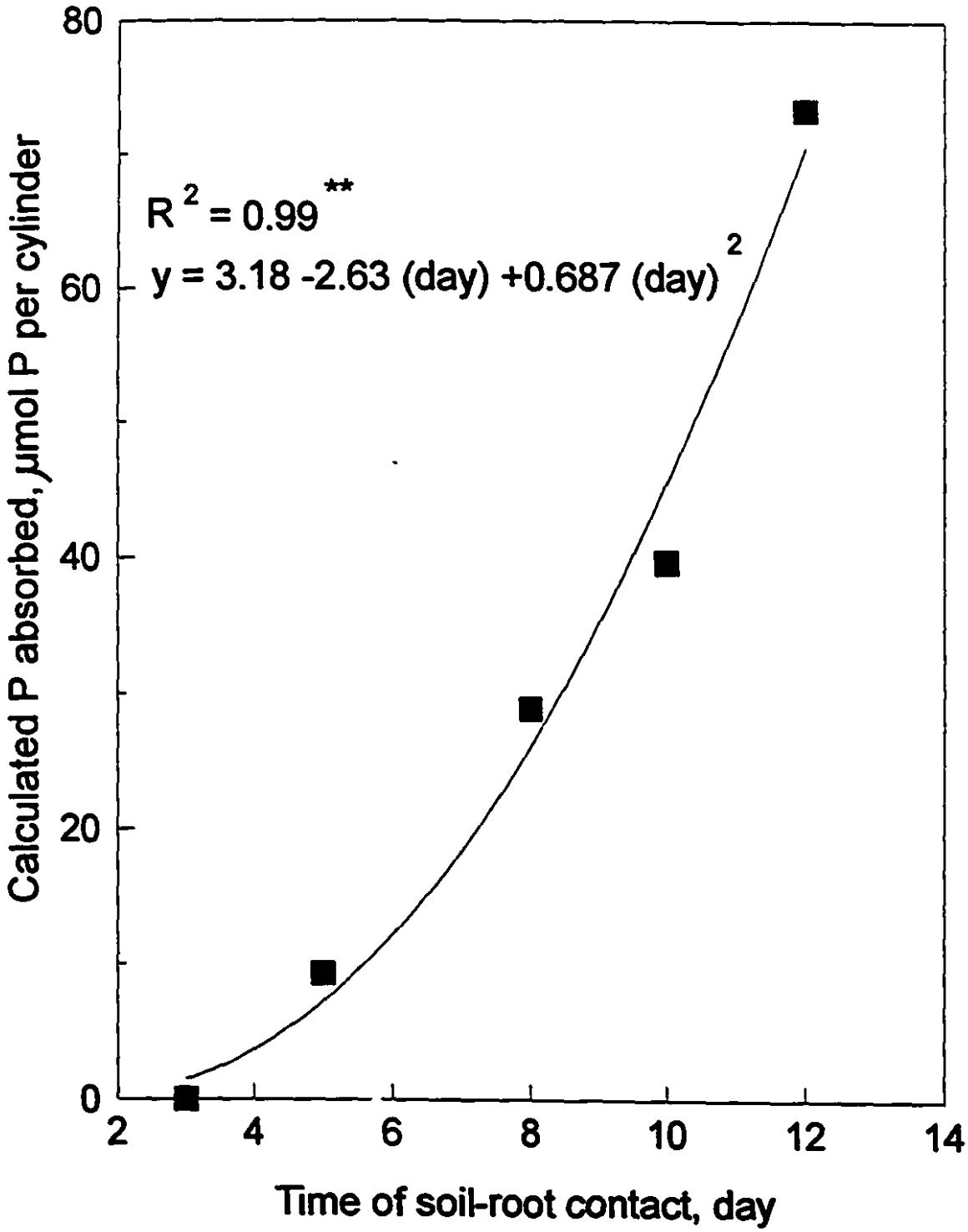

80
60
40
20
0
2
4
6
8
10
12
14
$R^2 = 0.99^{**}$
$y = 3.18 - 2.63\ (day) + 0.687\ (day)^2$
Calculated P absorbed, µmol P per cylinder
Time of soil-root contact, day

Figure 4. Changes in value with distance from the root plane of [a] total and residual P and [b] alkali soluble P on day three. Each point represents the mean of two replicates. Bars are standard errors.

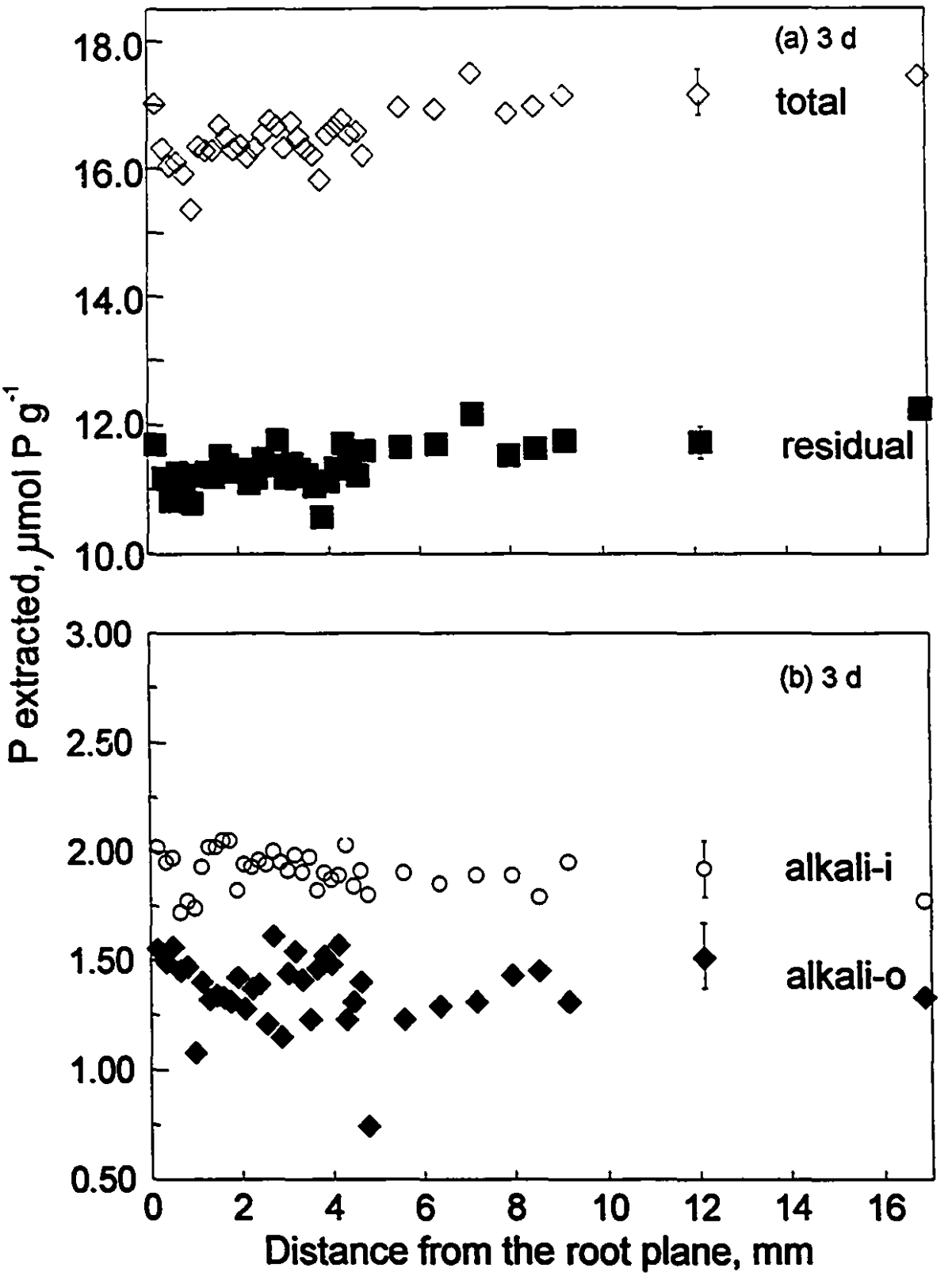

(a) 3 d
total
residual
18.0
16.0
14.0
12.0
10.0
(b) 3 d
alkali-i
alkali-o
3.00
2.50
2.00
1.50
1.00
0.50
P extracted, μmol P g-1
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 5. Changes in value with distance from the root plane of [a] total and residual P and [b] alkali soluble P on day 12. Each point represents the mean of two replicates. Bars are standard errors.

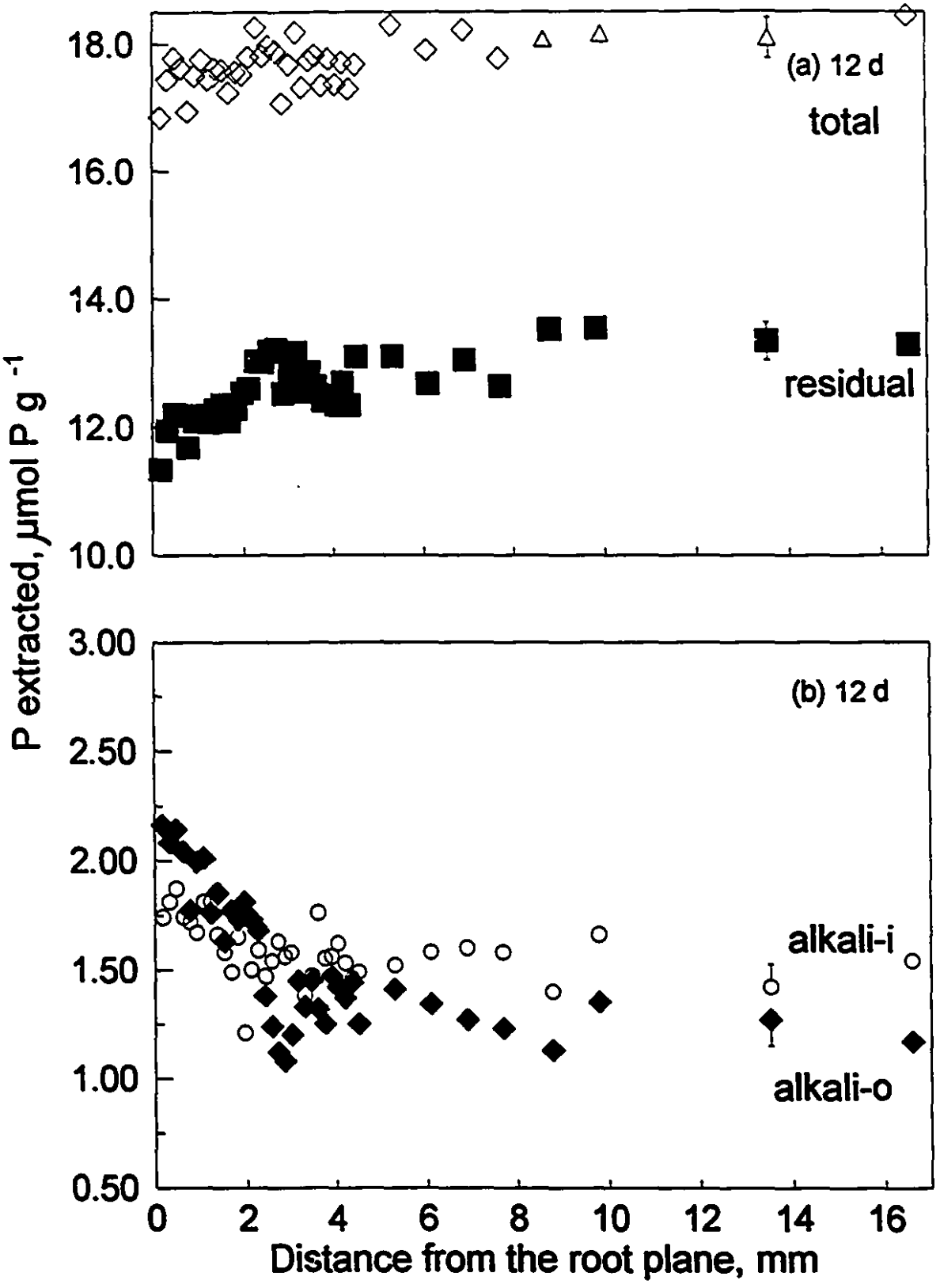

(a) 12 d
total
residual
(b) 12 d
alkali-i
alkali-o
P extracted, µmol P g -1
Distance from the root plane, mm
18.0
16.0
14.0
12.0
10.0
3.00
2.50
2.00
1.50
1.00
0.50
0
2
4
6
8
10
12
14
16

Figure 6. Changes in value with distance from the root plane of [a] acid soluble P and [b] pH on day three. Each point represents the mean of two replicates. Bars are standard errors.

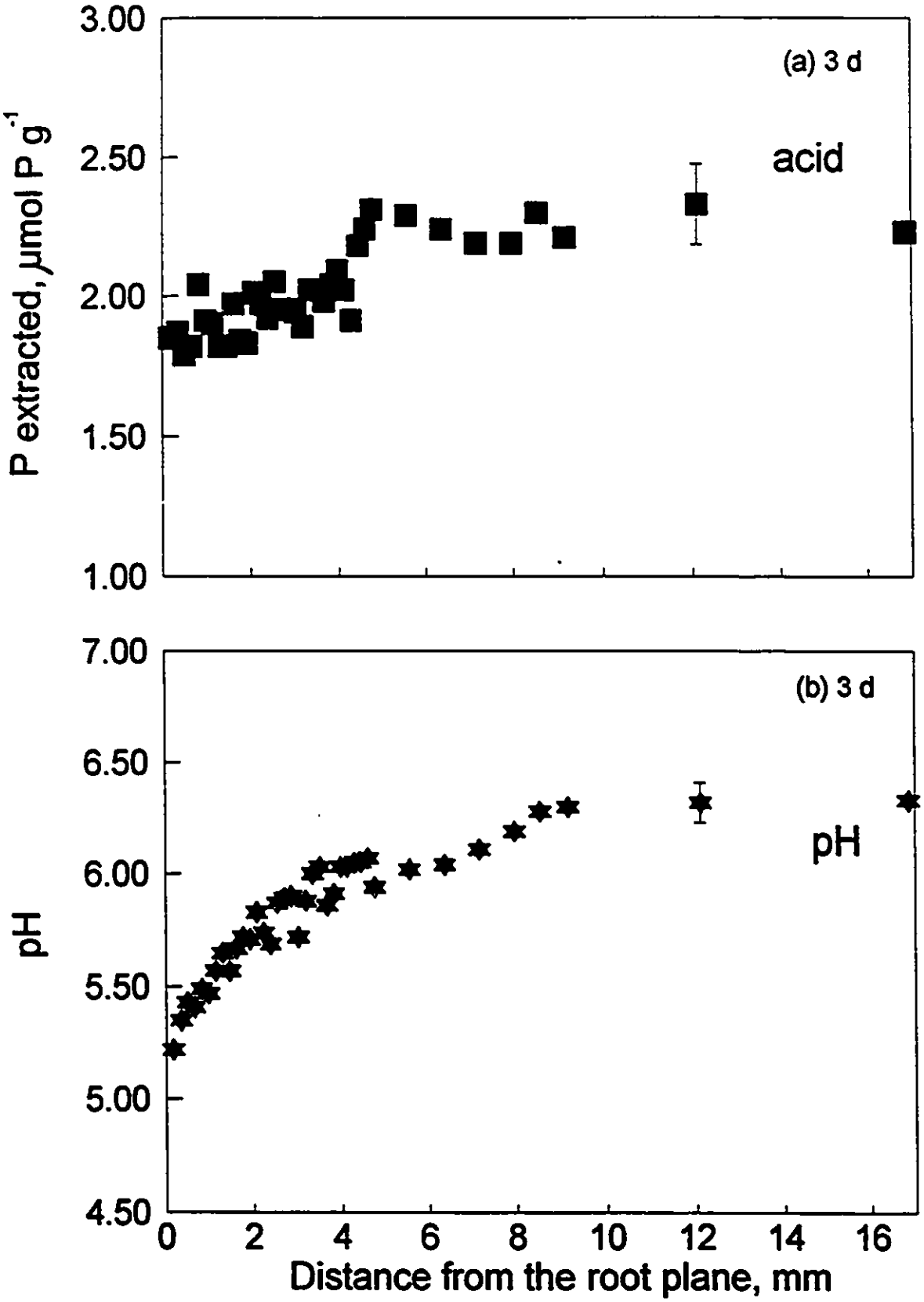

(a) 3 d
acid
P extracted, µmol P g-1
3.00
2.50
2.00
1.50
1.00
(b) 3 d
pH
7.00
6.50
6.00
5.50
5.00
4.50
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 7. Changes in value with distance from the root plane of [a] acid soluble P and [b] pH on day 12. Each point represents the mean of two replicates. Bars are standard errors.

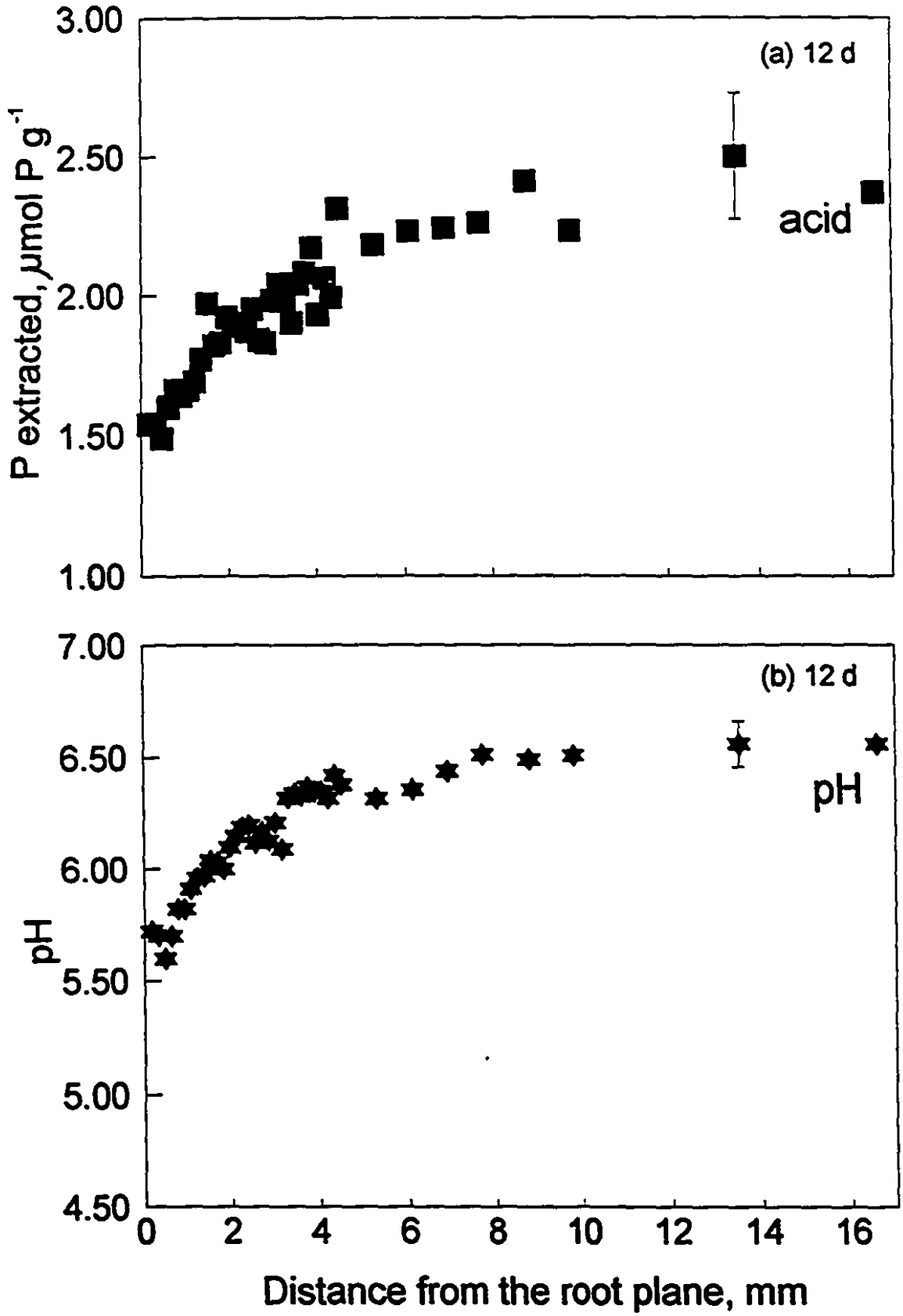

(a) 12 d
acid
P extracted, μmol P g-1
3.00
2.50
2.00
1.50
1.00
(b) 12 d
pH
7.00
6.50
6.00
5.50
5.00
4.50
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 8. Changes in P fractions in soil cylinders as compared to non-rhizosphere soil during soil-root contact. Positive values show an increase in a fraction as compared to non-rhizosphere soil. Each point represents the mean of two replicates. Bars are standard errors.

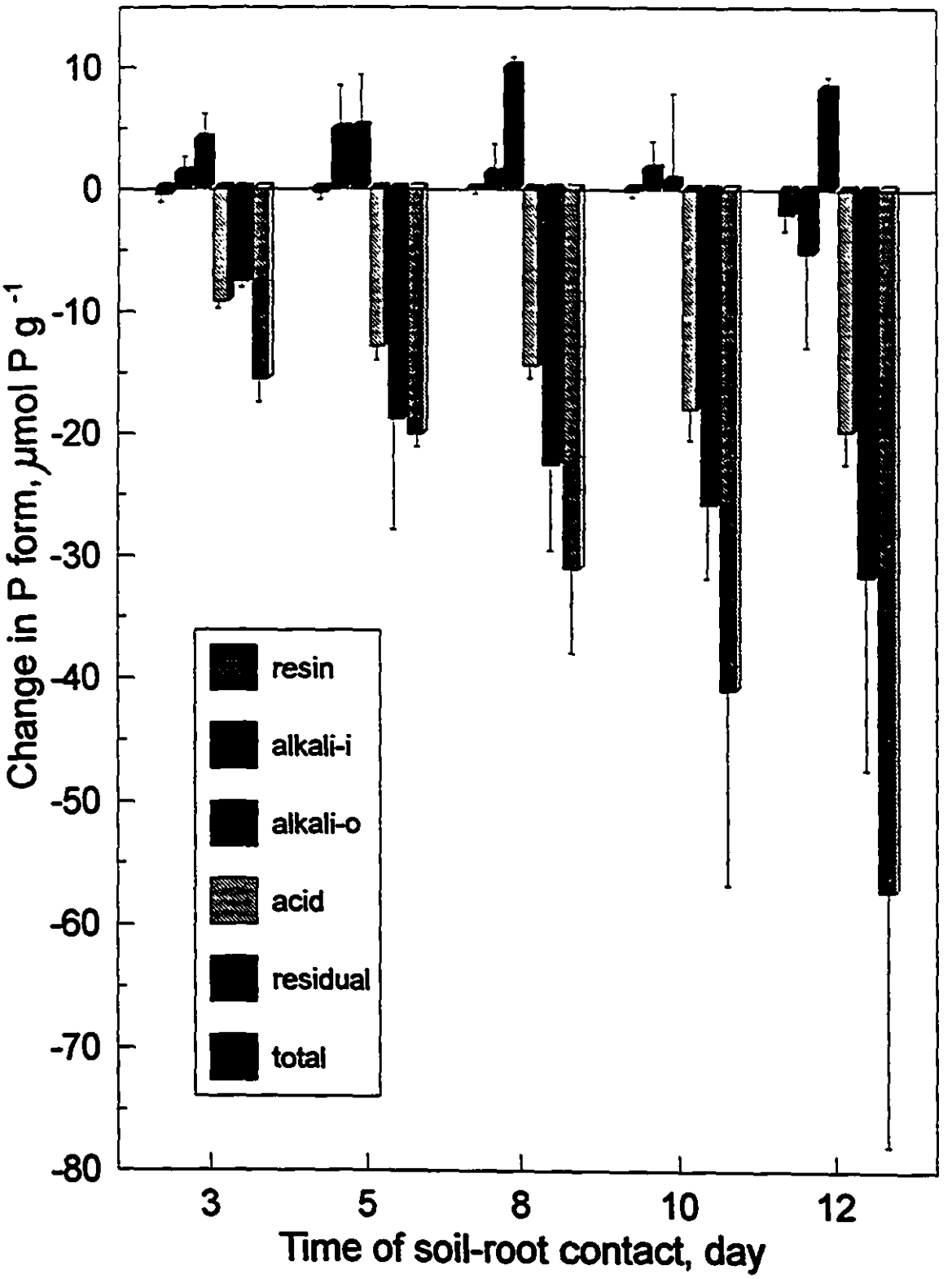

10
0
-10
-20
-30
-40
-50
-60
-70
-80
Change in P form, μmol P g-1
resin
alkali-i
alkali-o
acid
residual
total
3
5
8
10
12
Time of soil-root contact, day

Table 1. Properties of San Raphael loam soil used in experiment.

Property	Value
Texture	sandy loam
Aerobic pH (1 soil: 5 H_2O)	3.4
Anaerobic pH (1 soil: 5 H_20)	6.6
C g kg^{-1}	12.1
N g kg^{-1}	1.0
Extractable cations†, mmol kg^{-1}	
Ca^{2+}	16.6
Mg^{2+}	12.0
Fe^{2+}	174.6
Mn^{2+}	< 1
K^+	3.6
Na^+	5.8

† Measured in a 1 M NH_4OAc extract, (1 soil: 5 extract)

Table 2. Phosphorous fractions in the reduced soil slurry, equilibrated for two days, with and without 64 μmol P g^{-1} added as KH_2PO_4.

Fraction†	Without P	With P
	μmol g^{-1}	
Water-soluble	0.001 ± 0.001	0.03 ± 0.01
Resin	0.02 ± 0.01	0.42 ± 0.08
Alkali-inorganic	1.36 ± 0.10	25.3 ± 0.95
Alkali-organic	0.38 ± 0.04	30.4 ± 1.29
Acid	2.22 ± 0.09	16.7 ± 0.46
Residual	13.6 ± 0.93	14.1 ± 0.71
Total	17.6 ± 1.17	84.9 ± 3.50

† mean of five replicates

± standard error

Table 3. Plant dry matter (DM), P content and calculated P absorbed by plant at different sample intervals.

Sample interval	Plant DM	Plant P content	P absorbed
d	— g —	μmol g^{-1}	μmol $cylinder^{-1}$
3	2.78[a] ± 0.08	87[a] ± 1	0[a] ± 5
5	2.93[a] ± 0.19	89[ba] ± 4	9[a] ± 14
8	3.19[a] ± 0.40	94[bc] ± 1	29[a] ± 20
10	3.38[a] ± 0.42	95[bc] ± 2	40[a] ± 24
12	4.00[a] ± 1.00	97[c] ± 3	73[a] ± 54
Mean	3.26		30

Means followed by the same letter ([a-c]) in the same column are not significantly different at the $P < 0.05$ level. For plant P content LSD = 0.19 μmol P g^{-1}.

± standard error.

Table 4. Change in P fractions between bulk soil and rhizosphere soil (summed through 0 to 8 mm from the root plane) at sample intervals

sample	Resin	Alkali-i	Alkali-o	Acid	Residual	Total
d	----------	----------	μmol P per cylinder	----------	----------	----------
3	-0.52 ± 0.44	1.31 ± 1.28	4.10 ± 2.20	-9.19 ± 0.91	-7.59 ± 0.25	-15.6 ± 0.81
5	-0.33 ± 0.34	4.90 ± 4.84	5.05 ± 4.89	-12.9 ± 0.38	-18.8 ± 9.51	-20.1 ± 2.53
8	-0.04 ± 0.05	1.32 ± 2.21	10.0 ± 0.38	-14.4 ± 2.36	-22.5 ± 7.75	-31.0 ± 7.30
10	-0.21 ± 0.12	1.70 ± 3.61	0.73 ± 7.73	-17.9 ± 2.70	-25.8 ± 8.11	-41.0 ± 16.6
12	-2.00 ± 1.68	-5.18 ± 9.12	8.26 ± 1.62	-19.7 ± 3.32	-31.8 ± 16.2	-57.3 ± 22.8
Mean	-0.62	0.81	5.64	-14.8	-21.3	-33.0

Means of all P fractions were not significantly different with time

± standard error.

Table 5. Regression equations for change in P fractions through 0 to 8 mm from root plane versus sample period in days.

P fraction	Equation	R^2 value
Resin	$\Delta P = 2.72 + 0.874\ (day) - 0.0663\ (day)^2$	0.859^{NS}
Alkali-i	$\Delta P = -5.23 + 3.06\ (day) - 0.253\ (day)^2$	0.859^{NS}
Alkali-o	$\Delta P = 2.79 + 0.651\ (day) - 0.0309\ (day)^2$	0.040^{NS}
Acid	$\Delta P = -6.06 - 1.19\ (day) - 0.00463\ (day)^2$	0.958^{*}
Residual	$\Delta P = 2.35 - 4.22\ (day) + 0.124\ (day)^2$	0.941^{*}
Total	$\Delta P = -16.6 + 1.42\ (day) - 0.396\ (day)^2$	0.990^{**}

*, ** significant at the $P < 0.05$ and $P < 0.01$ levels, respectively.

^{NS} indicates not significant

CHAPTER V

SUMMARY OF RESULTS AND GENERAL CONCLUSIONS

The oxidation of α-naphthylamine, which is an indicator of root respiration reflected differences among rice cultivars and was a measure of root activity in response to P deficiency. Cultivars responded to P deficiency by reducing the pH of the rhizosphere but this was a response of the plant to imbalances in the cation-anion uptake ratio rather than a reaction to low P levels.

An apparatus and system were developed to study the oxidized rice rhizosphere in an anaerobic environment using intact plants. Methods for extracting Fe^{2+}, Fe^{3+} and measuring pH from anaerobic soil sections were developed.

Results from the rhizosphere study suggest large changes in the soil system induced by the plant root. Oxidation of Fe^{2+} by passive O_2 release from the roots added substantial amounts of H^+ to the system. Roots released additional H^+ due to excess intake of cations over anions (N is absorbed by rice roots as NH_4^+ rather than NO_3^- in an anaerobic system). The pH dropped 2 pH units within 3 mm of the plant root compared to the bulk reduced soil. Ferric Fe concentration increased 12-fold within 1 mm of the root surface. The range of Fe^{2+} depletion was broader than that of Fe^{3+} accumulation, but of a similar dimension to the pH decrease. Total quantities of Fe^{2+} oxidized and Fe^{3+} produced were approximately equal.

Oxygen fluxes from the root were calculated from the increases in total quantities of Fe^{3+} per cylinder. Values of O_2 fluxes ranged from 1.20 to 0.10 nmol O_2 dm^{-2} s^{-1} during the times between sampling. The decline with time in the calculated rate of O_2 loss may be due to an increase in O_2 consumption by aerobic

microbes in the rhizosphere and by a decrease in the rate of Fe^{2+} oxidation as the pH falls.

A method was developed to differentiate P fractions in anaerobic soil. Phosphorous fractions were: anion-exchangeable resin P; alkali-inorganic and -organic P in 0.1 M NaOH; acid soluble P in 1 M HCl + 1 M H_2SO_4; and residual P. Within the rice rhizosphere, profiles of P fractions and pH were obtained in reduced soil in contact with rice roots. Acid soluble P decreased in the soil within four mm of the root plane. The zone of depletion coincided with the zone of acidification. Alkali-inorganic and -organic P varied with distance from the root plane. Alkali-organic P showed an accumulation near the roots. Neither alkali-inorganic nor -organic extractable P was depleted by plant uptake. Mean values of total P depletions in the soil (57 $\pm$ 22 μmol per cylinder at 12 d) were less than mean values of P absorbed by the plants (73 $\pm$ 54 μmol g^{-1} at 12 d) but within standard error.

Rice plants growing in flooded soils may benefit from changes in solid-solution chemistry of P and Fe in the rhizosphere. These alterations are due to oxidation of Fe^{2+} by O_2 loss from rice roots. Oxidation of Fe^{2+} releases H^+ into the rhizosphere. Acidification of the rhizosphere is enhanced by H^+ extruded by rice roots to balance the excess cation over anion uptake. The depletion of acid soluble P near the root plane supports the idea that solubilization of P occurred along with acidification of the rhizosphere. The newly solubilized P is available for plant uptake. Rice cultivars that differ in root O_2 release and in cation-anion uptake

ratios may have different responses to P deficient soils.

SUGGESTIONS FOR FUTURE WORK

1. The amount of O_2 loss from rice roots in anaerobic soils affects the solid-solution chemistry of Fe and P, which in turn affects nutrient availability and toxicity problems. Future research should study the differences in root porosity and aerenchyma development in rice cultivars to determine if these plant characteristics affect cultivar response to P availability.

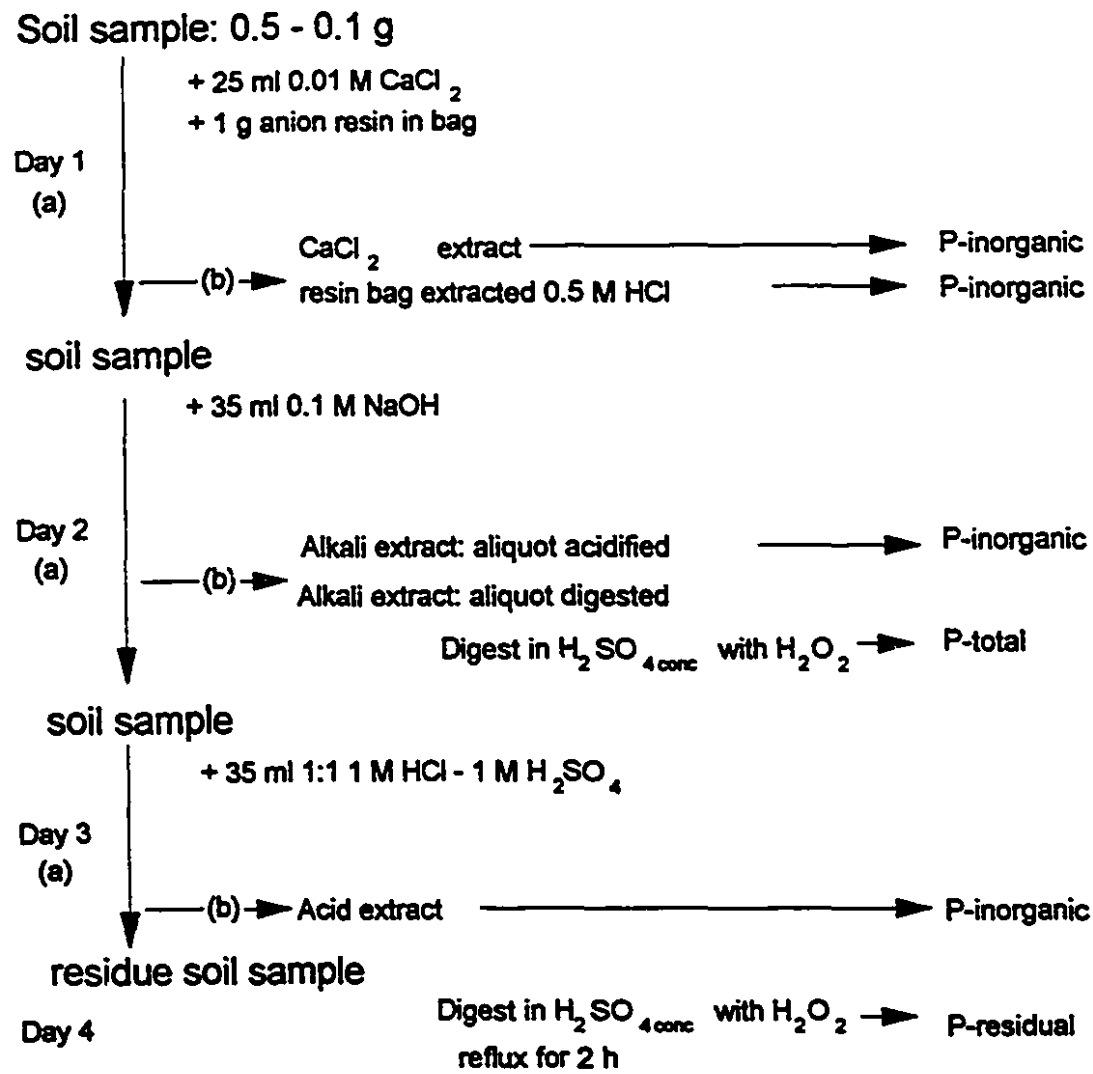
2. Lowland soils undergo alternate flooding and drying cycles during rice production. With the increase in intensive irrigated rice production, the duration of the drying cycles has dramatically decreased, which would alter the forms of Fe and P compounds found in the soil. Future research should study the effect of intensive rice production, oxidation-reduction regimes, on P fractions found in these soils.

3. The alkali-organic extractable P fractions in the rice rhizosphere varied throughout the experiment. The organic P fraction in anaerobic soils may be an important part of the P cycle. Future research should study the transformations of organic P in anaerobic soils and the effect of organic matter addition on P fractions in the rhizosphere.

4. In the rice rhizosphere, the microbial population is aerobic but surrounded by an anaerobic environment. The effect of the microbial population on the quantity of O_2 in the rhizosphere and subsequently Fe and P forms should be evaluated.

APPENDICES

Figure 1. Scheme for phosphorus fractionation in anaerobic soils



(a) Soil sample plus extractant were shaken end over end for 16 h at 60 r.p.m. and at 25°C

(b) Soil plus extractant centrifuged at 10,000 r.p.m. for 10 min before filtration

Figure 2. Changes in value with distance from the root plane of [a] total and residual P and [b] alkali soluble P on day five. Each point represents the mean of two replicates. Bars are standard errors.

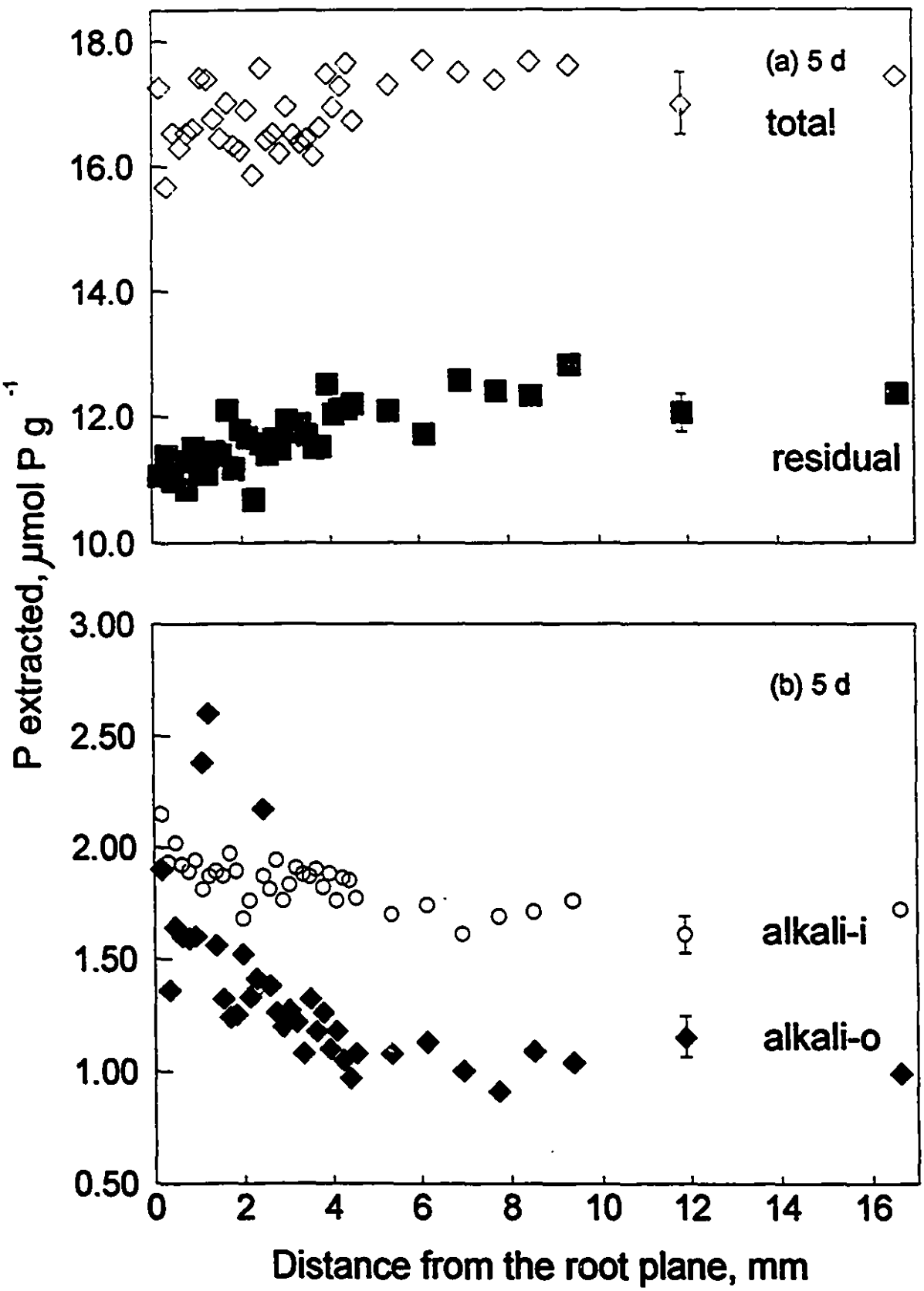

(a) 5 d
total
residual
18.0
16.0
14.0
12.0
10.0
(b) 5 d
alkali-i
alkali-o
3.00
2.50
2.00
1.50
1.00
0.50
0
2
4
6
8
10
12
14
16
P extracted, μmol P g-1
Distance from the root plane, mm

Figure 3. Changes in value with distance from the root plane of [a] total and residual P and [b] alkali soluble P on day eight. Each point represents the mean of two replicates. Bars are standard errors.

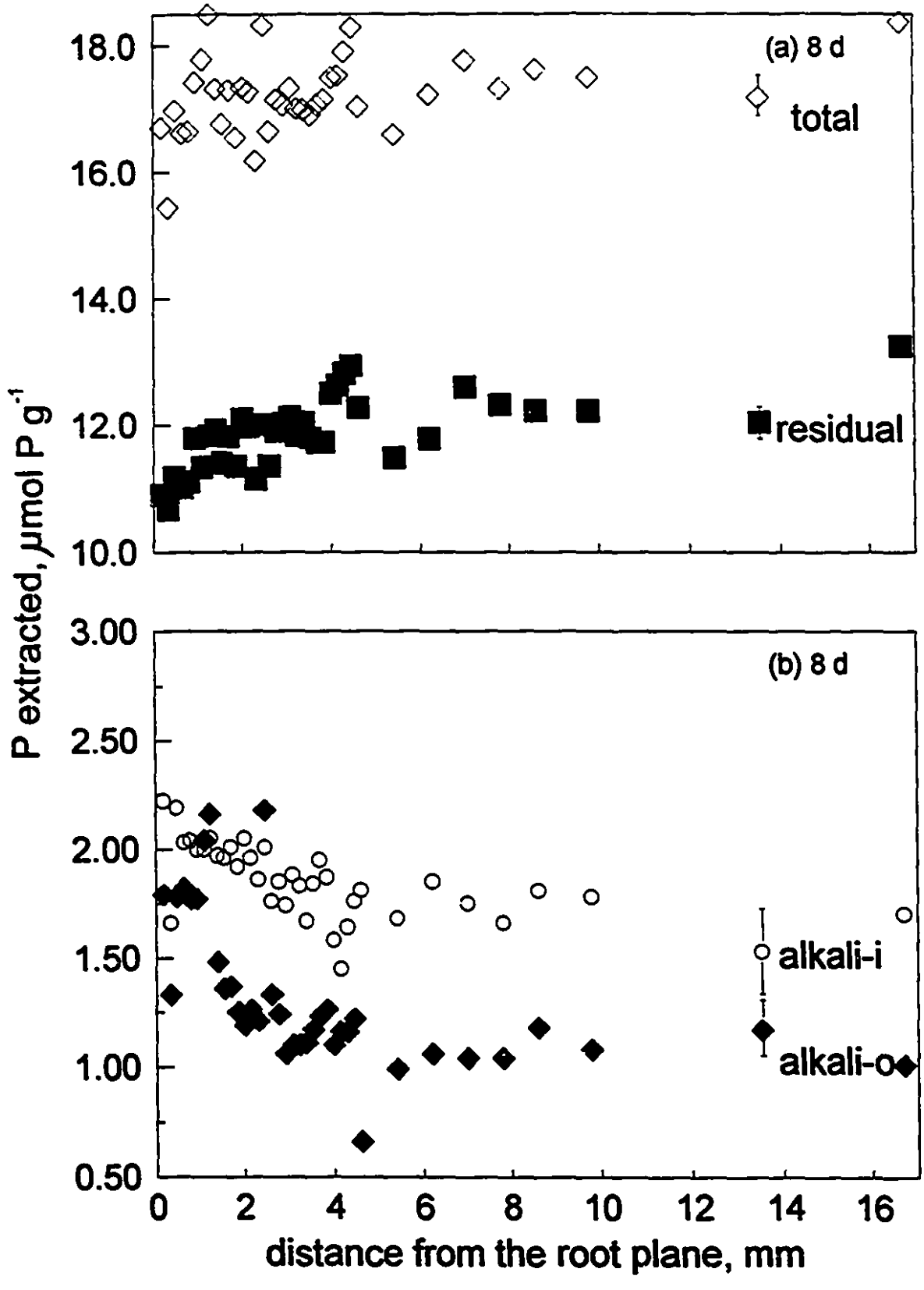

(a) 8 d
total
residual
18.0
16.0
14.0
12.0
10.0
(b) 8 d
alkali-i
alkali-o
3.00
2.50
2.00
1.50
1.00
0.50
P extracted, μmol P g-1
0
2
4
6
8
10
12
14
16
distance from the root plane, mm

Figure 4. Changes in value with distance from the root plane of [a] total and residual P and [b] alkali soluble P on day 10. Each point represents the mean of two replicates. Bars are standard errors.

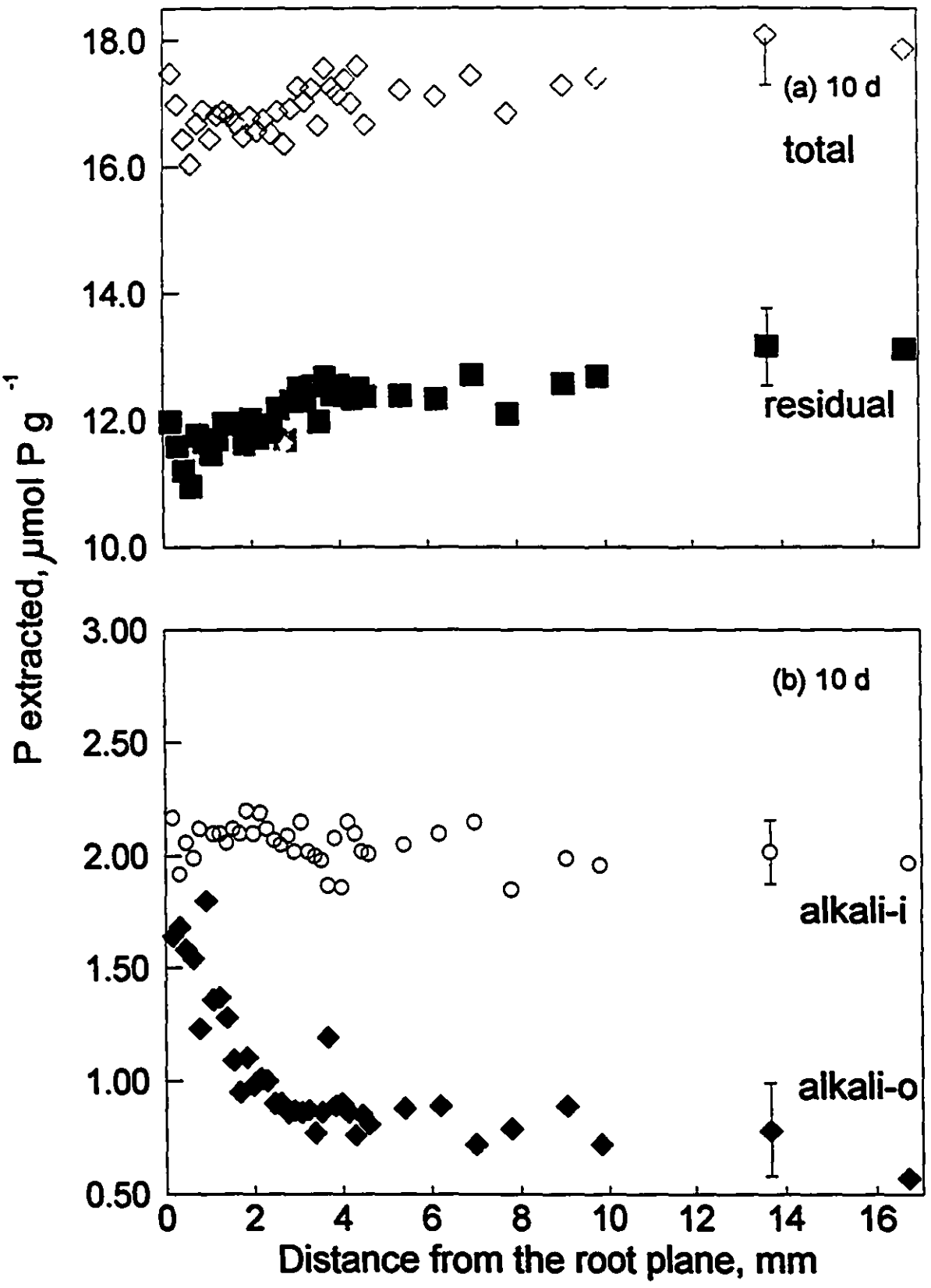

18.0
16.0
14.0
12.0
10.0
(a) 10 d
total
residual
3.00
2.50
2.00
1.50
1.00
0.50
(b) 10 d
alkali-i
alkali-o
P extracted, μmol P g-1
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 5. Changes in value with distance from the root plane of [a] acid soluble P and [b] pH on day five. Each point represents the mean of two replicates. Bars are standard errors.

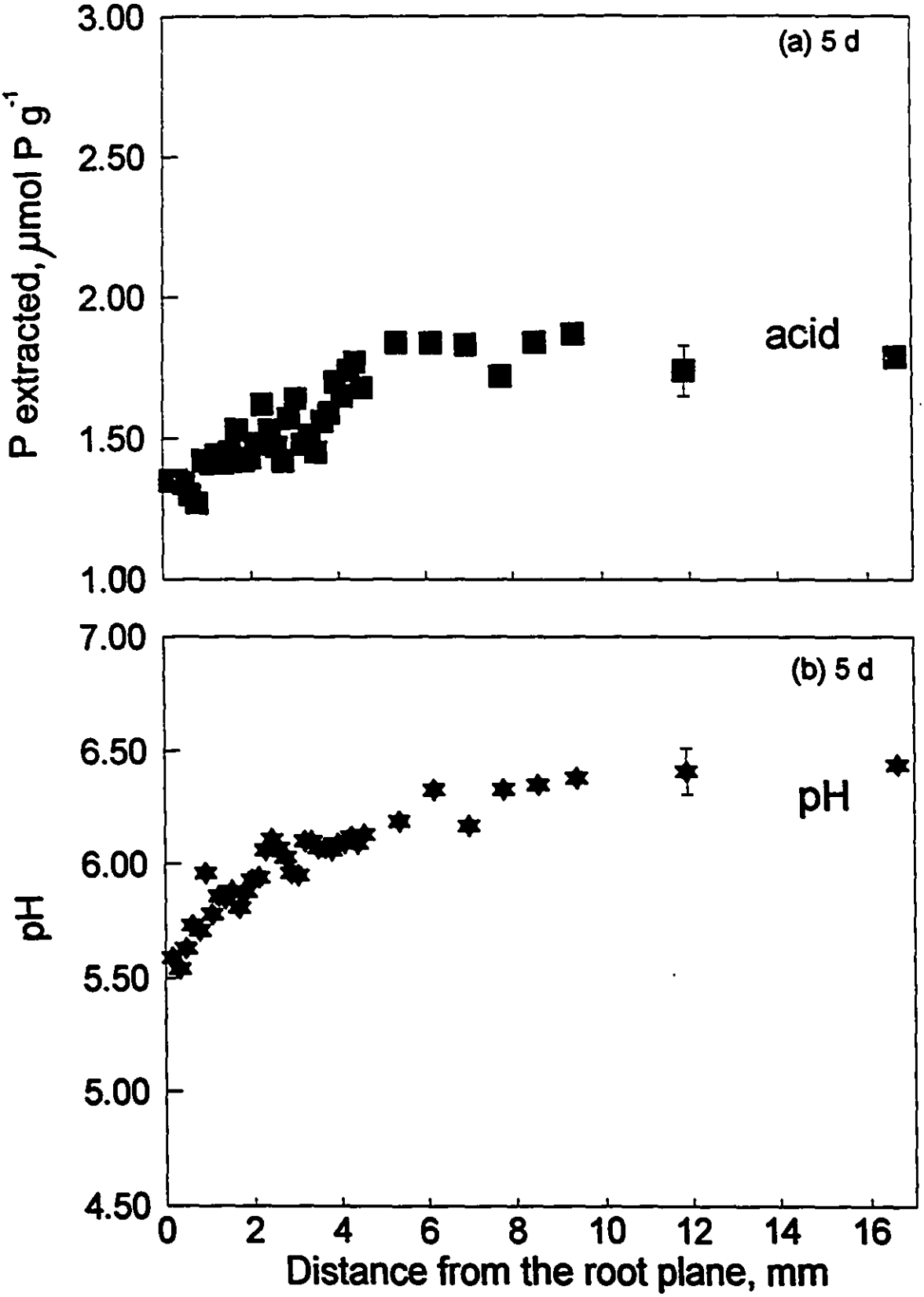

(a) 5 d
P extracted, μmol P g-1
3.00
2.50
2.00
1.50
1.00
acid
(b) 5 d
pH
7.00
6.50
6.00
5.50
5.00
4.50
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 6. Changes in value with distance from the root plane of [a] acid soluble P and [b] pH on day eight. Each point represents the mean of two replicates. Bars are standard errors.

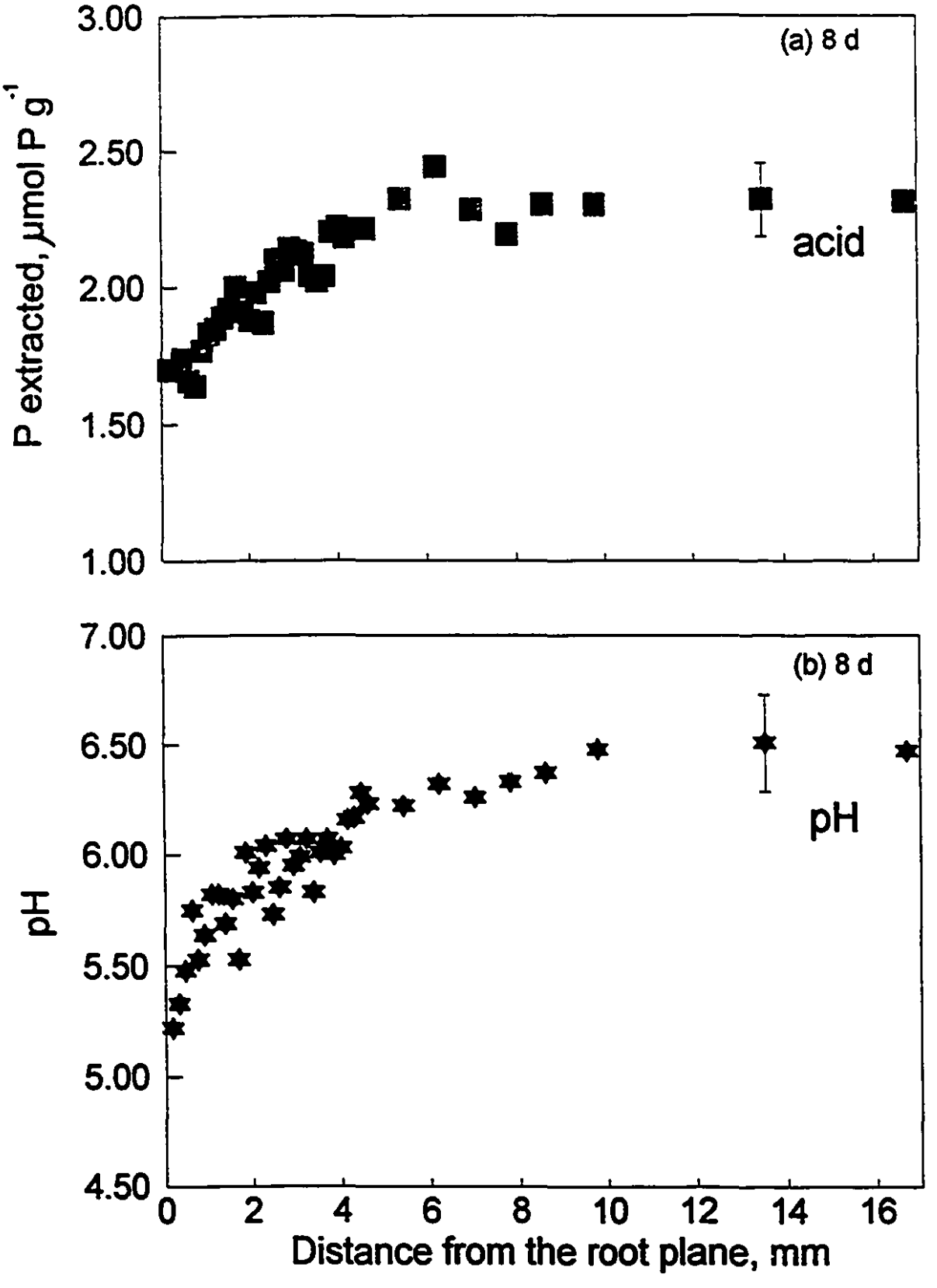

(a) 8 d
acid
P extracted, μmol P g-1
3.00
2.50
2.00
1.50
1.00
(b) 8 d
pH
7.00
6.50
6.00
5.50
5.00
4.50
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 7. Changes in value with distance from the root plane of [a] acid soluble P and [b] pH on day 10. Each point represents the mean of two replicates. Bars are standard errors.

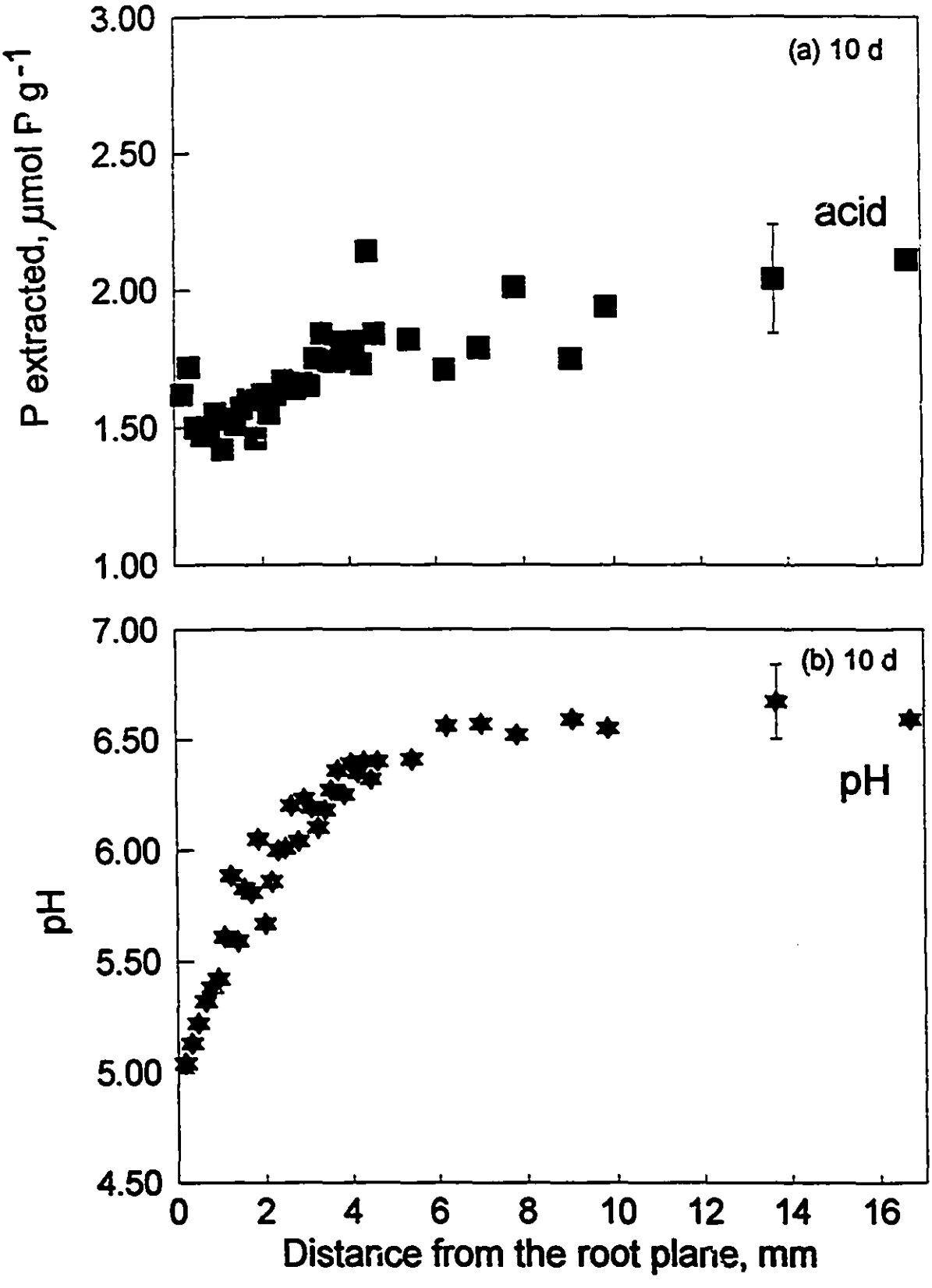

(a) 10 d
acid
P extracted, μmol P g-1
3.00
2.50
2.00
1.50
1.00
(b) 10 d
pH
pH
7.00
6.50
6.00
5.50
5.00
4.50
0
2
4
6
8
10
12
14
16
Distance from the root plane, mm

Figure 8. Change in acid soluble P through time.

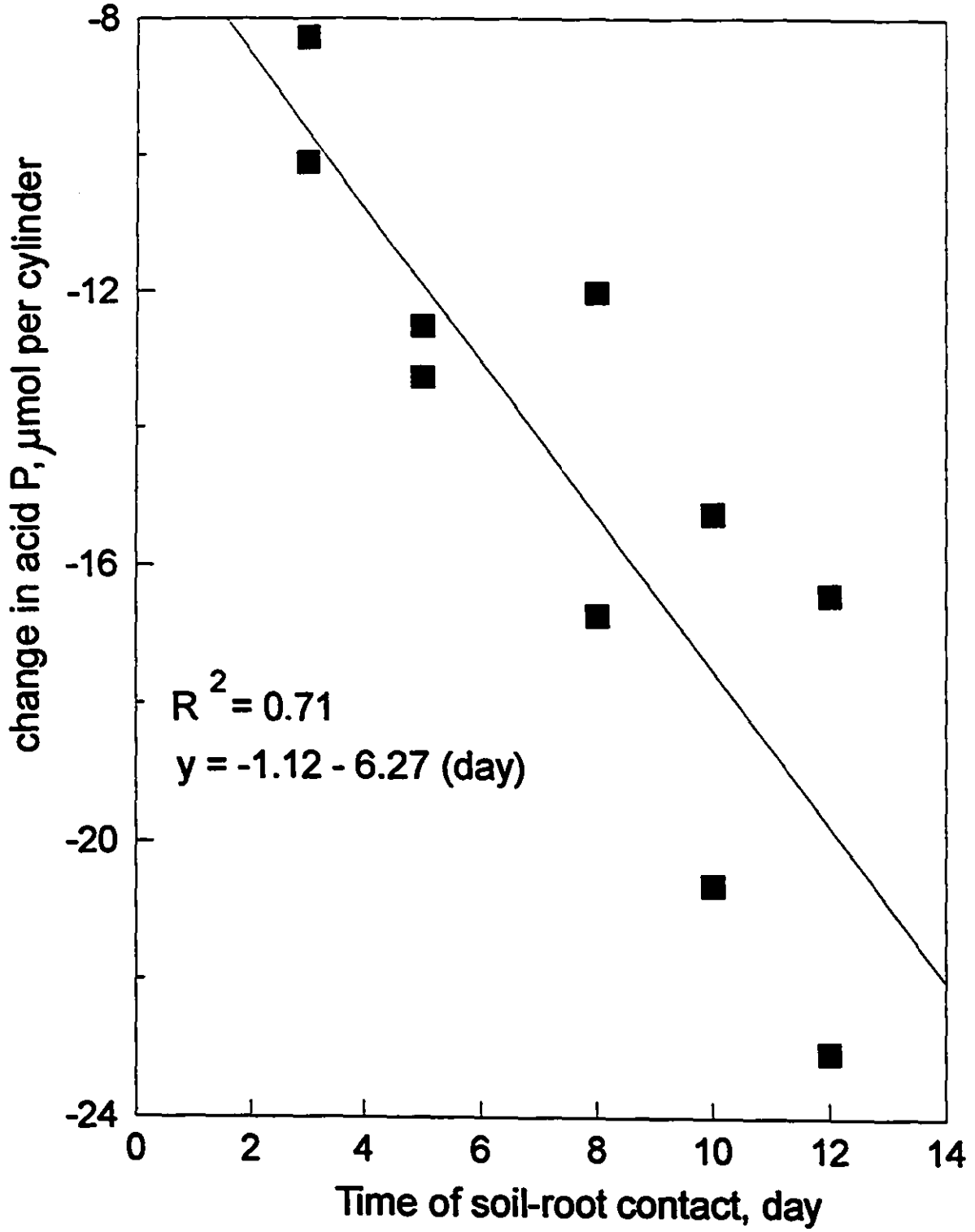

-8
-12
-16
-20
-24
0
2
4
6
8
10
12
14
change in acid P, µmol per cylinder
Time of soil-root contact, day
R 2 = 0.71
y = -1.12 - 6.27 (day)

Table 1. Composition of culture solution for plant growth and α-naphthylamine oxidation study

Element	Concentration
	mg dm^{-3}
N†	40
K	40
Ca	40
Mg	40
Mn	0.5
Mo	0.05
B	0.2
Zn	0.01
Cu	0.01
Fe	2

† N was added as NH_4NO_3

Table 2. Total plant dry weight[†] and Fe concentration in tissue

Sampling interval	Replicate 1 plant wt.	Fe	Replicate 2 plant wt.	Fe
d	g	μmol g^{-1}	g	μmol g^{-1}
3	2.86	72	2.70	67
5	3.12	68	2.73	75
8	3.59	58	2.79	90
10	3.80	74	2.95	95
12	4.99	120	3.00	110

[†] all four plants per cell combined for analysis, includes both root and shoot

Table 3. Total plant nutrient content at sampling intervals after contact with anaerobic soil

Sampling interval	plant weight	N	P	K	Ca	Mg
d	g	% wt./wt.				
3	2.78 ± 0.08[†]	2.61a ± 0.09	0.263a ± 0.012	1.03 ± 0.19	0.20 ± 0.01	0.16ab ± 0.11
5	2.93 ± 0.19	2.84b ± 0.02	0.285ba ± 0.014	1.18 ± 0.17	0.26 ± 0.01	0.23a ± 0.10
8	3.19 ± 0.40	2.94b ± 0.01	0.289bc ± 0.011	1.18 ± 0.18	0.29 ± 0.02	0.34abc ± 0.40
10	3.38 ± 0.42	2.96b ± 0.04	0.292bc ± 0.015	0.94 ± 0.09	0.29 ± 0.03	0.36bc ± 0.05
12	4.00 ± 1.00	3.01b ± 0.01	0.302c ± 0.011	1.00 ± 0.01	0.32 ± 0.02	0.40c ± 0.04

[†] standard deviation of the mean.
Mean of two replicates (four plants per cylinder).
Means in the same column with the same letters are not significantly different at the $P < 0.05$ level by the LSD test.
LSD = 0.19 for N, 0.0196 for P and 0.13 for Mg.
Columns without letters were not significantly different among days.

Table 4. Total quantities of Fe^{2+} consumed and Fe^{3+} formed in each cylinder at different sample times, with and without plants†

with plant roots		
Sample interval	Fe^{2+}	Fe^{3+}
d	mmol per cylinder	
3	0.73 ± 0.40‡	0.79a ± 0.24
5	1.28 ± 0.04	0.94a ± 0.07
8	2.02 ± 0.07	1.78b ± 0.13
10	1.41 ± 0.34	1.36c ± 0.08
12	1.86 ± 0.07	1.76b ± 0.09

without plant roots		
Sample interval	Fe^{2+}	Fe^{3+}
d	mmol per cylinder	
3	0.14 ± 0.01	0.16 ± 0.05
5	0.15 ± 0.08	0.14 ± 0.06
8	0.34 ± 0.08	0.39 ± 0.09
10	N/A	N/A
12	0.54 ± 0.25	0.47 ± 0.21

† Mean of two replicates.
‡ standard deviation of the mean.
Means in the same column with the same letters are not significantly different at the $P < 0.05$ level by the LSD test. LSD = 0.27 for Fe^{3+}.
Columns without letters were not significantly different among days.

Table 5. Estimated quantities of total H^+ formed near the root plane and calculated quantities of H^+ released from Fe oxidation and from roots to balance excess uptake of cations over anions.

Sample interval	H^+ formed in oxidation	H^+ released from ion imbalance	Total H^+ formed†
d	——— mmol per cylinder ———		
3	1.58a ± 0.48‡	0.27 ± 0.10	2.14 ± 0.03
5	1.88b ± 0.14	1.31 ± 0.21	2.15 ± 0.10
8	3.55c ± 0.25	1.74 ± 0.54	3.79 ± 0.14
10	2.72c ± 0.16	2.36 ± 0.49	3.06 ± 0.40
12	3.52c ± 0.18	3.99 ± 0.56	2.47 ± 1.19

† estimated from the pH profile curve for each day.
‡ standard deviation of the mean.
Mean of two replicates.
Means in the same column with the same letters are not significantly different at the $P < 0.05$ level. LSD = 0.64 for H^+ formed in oxidation.
Columns without letters were not significantly different among days.

Table 6. Calculated O_2 fluxes across the root-plane surface averaged over the times between samplings

Sample interval	O_2 flux
d	nmol dm^{-2} s^{-1}
0-3	1.59 + 0.07 ..
3-5	1.67 ± 0.06
5-8	1.65 ± 0.06
8-10	0.92 ± 0.22
10-12	1.02 ± 0.10

Mean of two replicates.

Means were not significantly different among days.