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Direct Acquisition of Organic Nitrogen by Crops

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Abstract

Growth response to organic nitrogen application was observed among various crops. We used rice bran mixed with rice straw as organic nitrogen source with a C/N ratio of 20. Nitrogen was not readily released from the organic matter because of the high C/N ratio. Organic nitrogen was applied with chemical fertilizers in an upland field to various crops such as upland rice, maize, potato, sugar beet and soybean. Inorganic nitrogen level in fallow soil was lower until the middle of the growth stage when organic nitrogen and chemical N-P-K fertilizers were applied, compared to the application of chemical fertilizers, only. In contrast to the inorganic nitrogen level in soil, upland rice grew better with organic nitrogen than chemical fertilizer, and took up more nitrogen than other crops during the first 100 days after amendment with organic nitrogen. These results can be attributed to 2 factors. (1) Higher mineralization rate in the rhizosphere of upland rice compared with other crops. Upland rice may enhance nitrogen mineralization in soil by the secretion of some enzymes such as protease and/or materials promoting microorganism activity. (2) Upland rice has a greater ability to take up organic nitrogen directly. If a crop is capable of taking up organic nitrogen in the form of amino acids, peptides and protein, the crop could absorb nitrogen with less competition for nitrogen with microorganisms compared with a crop which absorbs nitrogen mainly as nitrate. The protease activity in the soil planted with maize and sugar beet was higher than that in the soil planted with upland rice and in fallow soil. These results show that the enhancement of nitrogen mineralization is not essential for upland rice. In relation to hypothesis (2), upland rice took up more amino acids than maize in a solution culture containing some kinds of amino acids. The amino acid and protein contents in the soils from the rhizosphere and non-rhizosphere zones of upland rice (hereafter referred to as "rhizosphere and non-rhizosphere soils") were lower than those in the case of maize, soybean and fallow soil. We also observed that upland rice took up nitrogen before mineralization based on a ^{15}N-labelled rice bran application test. These results suggest that upland rice may take up organic nitrogen preferentially compared with other crops. We examined the nitrogen uptake mechanism of upland rice and maize. Upland rice takes up nitrogen preferentially in the organic form rather than as nitrate which is the favorite form for maize. Endocytosis is one of the mechanisms for organic nitrogen uptake by upland rice.

Discipline: Soils, fertilizers and plant nutrition
Additional key words: maize, nitrogen uptake, rice bran, upland rice

Introduction

Sustainability of agriculture became a major issue of global concern during this decade. Agronomic practices aimed at reducing the dependence on inputs such as chemical fertilizers can contribute to sustainability. Nitrogen is the most limiting and commonly applied nutrient for crop production. The development of nutrient-responsive cultivars especially during the past 3 decades led to an intensive use of nitrogen fertilizers in many agricultural systems. Environmental and economic problems associated with such a practice have, however, generated an interest in alternative management systems, including the exploitation of beneficial biological functions and substitution of chemical fertilizers with farm-generated products.

Application of organic matter has long been known to improve the soil physical properties

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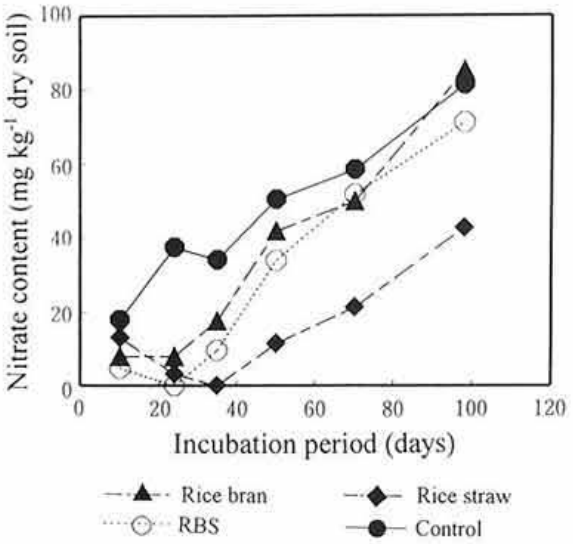

Fig. 1. Changes in the amount of nitrogen released from soil amended with rice bran and rice straw singly or in combination (RBS) at 30°C

besides providing several nutrients including nitrogen. Nitrogen fertility of soils is determined by the amount of inorganic nitrogen released from soils during incubation in the laboratory[4]. In fact, the nitrogen fertility measured by this method is positively correlated with the nitrogen uptake by plants[10]. There are several arguments, however, against the use of this method. The amount of nitrogen uptake by plants does not always correspond to the amount of nitrogen released from soils, but depends on plants under the same growth conditions. This does not occur if plants take up inorganic nitrogen formed from organic nitrogen in soils through natural mineralization. It is thus suggested that some crops take up nitrogen in a special form.

Nitrogen uptake response of crops to organic nitrogen application

Growth response to organic nitrogen application has been studied in upland rice and maize in a field

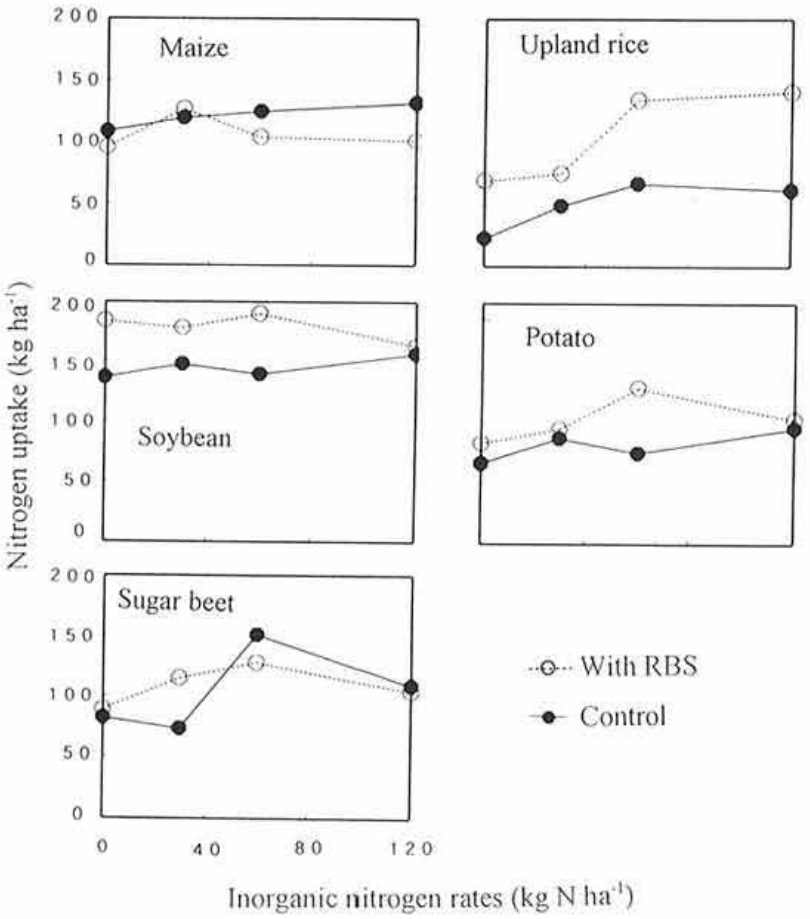

Fig. 2. Nitrogen uptake response of various crops to organic nitrogen at various inorganic nitrogen rates applied as ammonium sulfate under field conditions

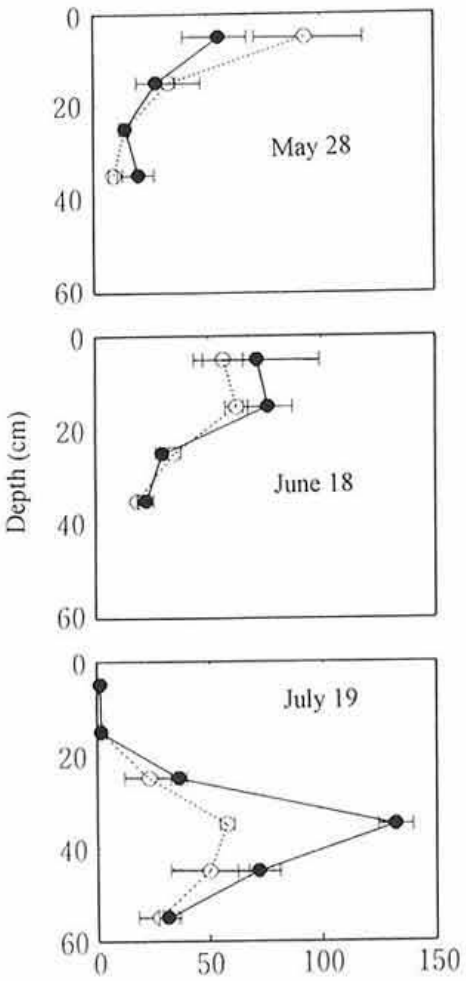

Fig. 3. Changes in inorganic nitrogen content at each depth of the fallow plot amended with ammonium sulfate at 80 kg ha^{-1} nitrogen rate

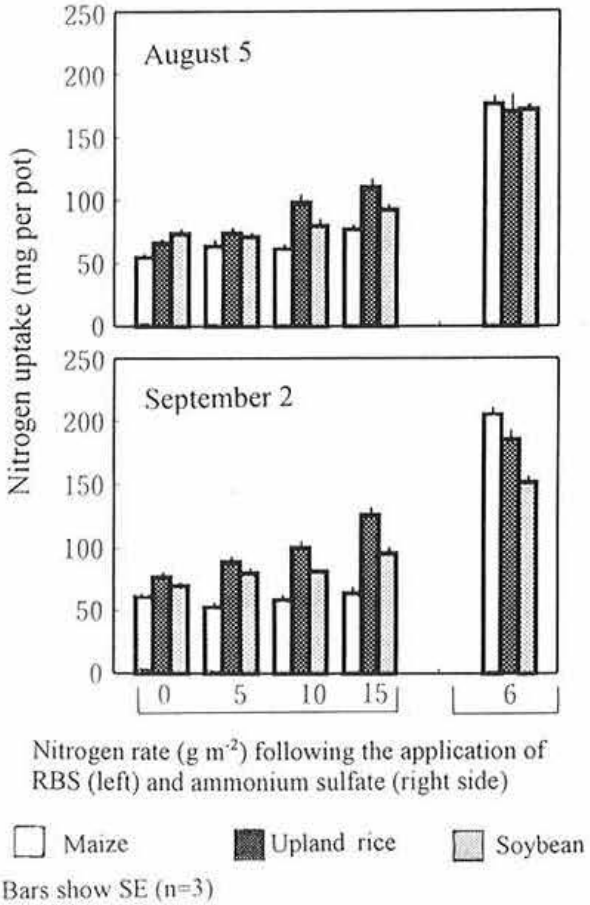

Fig. 4. Nitrogen uptake by various crops at two growth stages following the application of RBS and ammonium sulfate in a pot experiment in 1992

experiment using an inorganic nitrogen source (ammonium fertilizer) and an organic nitrogen source, consisting of a mixture of rice bran and rice straw as amendments. Fig. 1 shows the nitrogen mineralization pattern from soils with rice bran and/or rice straw application.

1) Field experiment

In 1992, maize (*Zea mays* L. var. DK250), potato (*Solanum tuberosum* L. var. Toyoshiro), soybean (*Glycine max* Merr. var. Tachinagaha), sugar beet (*Beta vulgaris* L. var. Beetmonobar) and upland rice (*Oryza sativa* L. var. Toyohatamochi) were grown in an Andosol (41.7 g C kg^{-1}, 3.4 g N kg^{-1} dry soil) field at the National Institute of Agro-Environmental Sciences, Tsukuba, Japan. All the cultivations were performed under upland field conditions. Organic nitrogen was supplied as a 4:1 mixture of rice bran and straw (RBS). The C/N ratio of this mixture was 20. In 1993, only maize and upland rice were cultivated to confirm the reproducibility.

Nitrogen uptake by upland rice, soybean and potato supplied with organic nitrogen was higher than that of the control (Fig. 2). However, the amount of inorganic nitrogen in the fallow plot amended with organic nitrogen was lower than in the control (Fig. 3). These facts indicate that nitrogen uptake by some crops did not reflect the nitrogen levels in soil, and that they displayed a nitrogen uptake ability in the presence of organic nitrogen sources[11].

2) Pot experiment

Pot experiments were conducted to study this

phenomenum more precisely by avoiding the effect of root spread. In 1992 and 1994, maize, soybean (non-nodulated cultivar, var. T201) and upland rice were cultivated in pots (vol 3.8 L) containing approximately 3 kg dry soil (Andosol). Organic nitrogen was supplied with RBS at a rate of 0.1 g kg^{-1} soil.

Nitrogen uptake by upland rice was higher than that by the other crops, and showed a positive relationship with the amount of organic matter applied (Fig. 4). In contrast, maize took up nitrogen actively in the ammonium sulfate plot, indicating that the nitrogen uptake response to organic nitrogen does not depend on the root spread but on crop properties.

We proposed 2 hypotheses for the higher nitrogen uptake of upland rice compared with maize as follows: Upland rice (1) accelerates nitrogen mineralization from organic nitrogen, by secreting some enzymes[9] or by stimulating the activity of microorganisms[3], and (2) takes up nitrogen as protein, amino acids and ammonium preferentially with little competition with microorganisms before mineralization[12].

Factors for differences in nitrogen uptake among crops

1) Differences in nitrogen uptake derived from ^{15}N-labelled rice bran among crops

Upland rice, maize, and soybean were cultivated with ^{15}N-labelled rice bran in pot trials. The crops and soils were sampled to determine the nitrogen and ^{15}N contents at 3 stages. The total nitrogen content in the plants and inorganic nitrogen content in the soils were determined by a calorimetric method following Kjeldahl digestion and KCl extraction, respectively. The content of ^{15}N was measured by emission spectrometry.

The ^{15}N content of upland rice was highest among other crops (Table 1), suggesting that upland rice is more likely to take up nitrogen in the form of ammonium, amino acids and peptides than nitrate which would reduce ^{15}N concentration in a plant, because nitrate would be diluted by the nitrate present in soil[15]. This assumption was supported by the fact that no differences were detected in the ammonium chloride plot.

2) Differences in protease activity in rhizosphere among crops

Protease activity in the soil planted with crops was measured to determine how much the so-called "rhizosphere effect" contributed to the nitrogen uptake. We cultivated upland rice, maize and soybean (var. T201) in small pots (5 × 5 × 5 cm) containing Andosol in 1995. We sampled the soils in the rhizosphere and non-rhizosphere zones separately at

Table 1. ^{15}N concentration (atom%) of nitrogen taken up by various crops using ^{15}N-labelled RBS and ammonium chloride

^{15}N-labelled source		Days after sowing		
		56	69	82
RBS	Upland rice	1.44 ± 0.04	1.53 ± 0.01	1.54 ± 0.02
	Maize	1.39 ± 0.06	1.36 ± 0.01	1.38 ± 0.03
	Soybean	1.26 ± 0.03	1.33 ± 0.03	1.38 ± 0.01
Ammonium chloride	Upland rice	3.11 ± 0.10	3.05 ± 0.02	3.02 ± 0.04
	Maize	3.06 ± 0.06	3.09 ± 0.03	2.90 ± 0.04
	Soybean	2.80 ± 0.06	2.90 ± 0.02	2.89 ± 0.02

Table 2. Protease activity in pot soils planted with various crops in RBS plot at original pH

	Protease activity (μmol h^{-1} g^{-1} dry soil)			
	Caseine method		Z-phe-leu method	
Crops	26 days	34 days	26 days	34 days
Upland rice	1.62	1.64	1.57	1.78
Maize	2.67	2.11	1.56	1.96
Soybean	1.52	1.78	1.71	1.90
Fallow	2.19	1.31	1.48	1.67

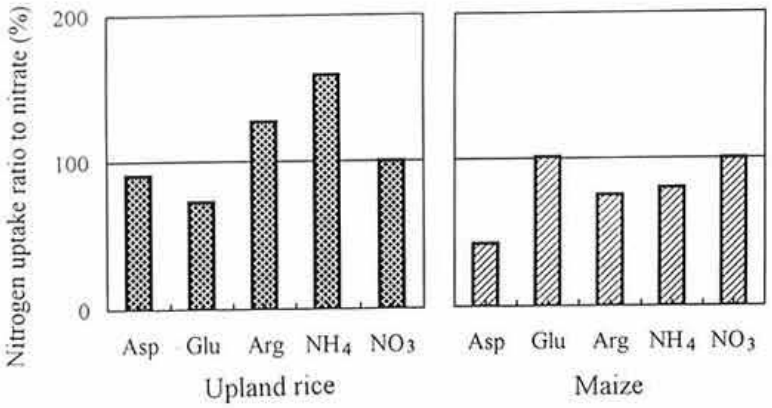

Fig. 5. Nitrogen uptake efficiency in the form of ammonium and amino acids in upland rice and maize expressed as ratio to nitrate uptake (=100) in solution culture

26 and 34 days after sowing, and measured the protease activity of these soils using both casein and Z-phe-leu as substrates[5].

Table 2 shows the protease activity in whole pot soil for each crop including rhizosphere and non-rhizosphere soils. The activity in the case of maize was higher than that of upland rice, while in the case of soybean an intermediate activity between these 2 crops was detected for both substrates. These results suggest that the rhizosphere effect involved in nitrogen mineralization is not related to the high nitrogen uptake by upland rice[14].

3) Amino acid application in solution culture

Rice and maize seedlings were initially grown for 30 days in perlite pots without any addition of nutrients. After sterilization in an ampicillin solution, the seedlings were supplied with a nutrient solution consisting of 1 M nitrogen solution of nitrate, ammonium, aspartate, glutamate and arginine, individually. These amino acids are considered to be the major components of rice bran protein[8].

Nitrogen uptake efficiency of ammonium, aspartate and arginine in upland rice was much higher than in maize with the exception of glutamate (Fig. 5). These results suggest that upland rice could directly take up amino acid nitrogen more effectively than maize.

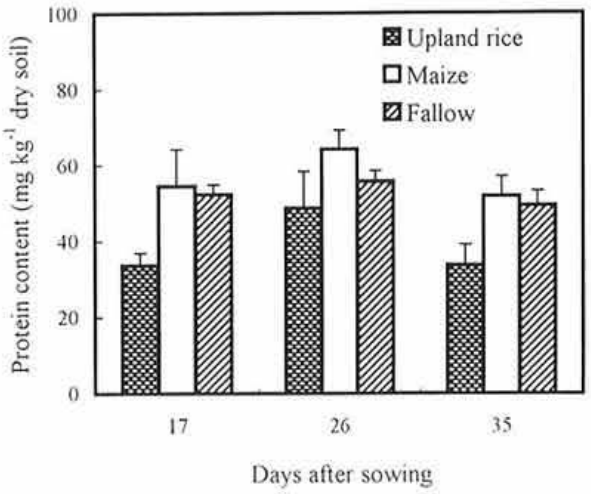

Fig. 6. Protein content in rhizosphere soil of upland rice and maize in a pot trial with RBS
Bars indicate SE (n=3).

4) Contents of various forms of nitrogen in pot soil

Upland rice and maize were grown in an Andosol. The contents of protein in the rhizosphere soils

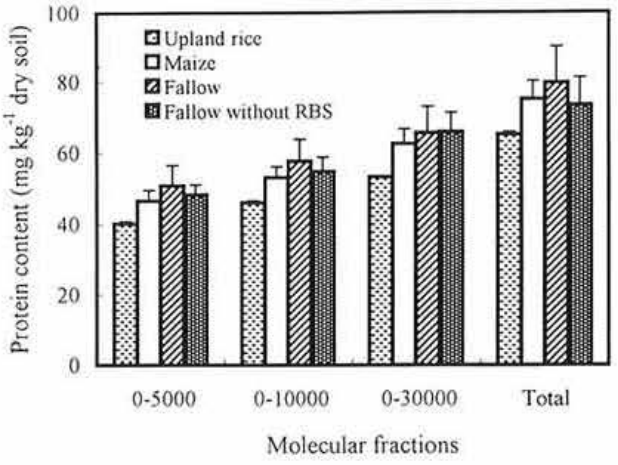

Fig. 7. Content of protein fractionated into molecular fractions followed by extraction with phosphate buffer in rhizosphere and fallow soil amended and not amended with RBS at 62 days after sowing
Bars indicate SE (n=3).

of upland rice at all sampling dates after sowing were the lowest among other crops (Fig. 6). The fractionated protein contents of all the rhizosphere soils at 62 DAS were lower than those of fallow soil (Fig. 7). The protein content in fallow soil without RBS was lower than that in fallow soil with RBS, but still higher than that in the rhizosphere soil of crops. These facts indicate that upland rice took up protein over a wide range of molecular weights from the soil, exceeding the amount of protein derived from RBS. As the protease activity in the rhizosphere soil of upland rice was lower than that of maize, the lower concentration of protein in the rhizosphere soil of upland rice did not result from the hydrolysis of protein by the rhizosphere protease[13].

Differences in nitrogen uptake between upland rice and maize

Fig. 8 shows the nitrogen dynamics in the rhizosphere soil and differences in nitrogen uptake between upland rice and maize. In this scheme, protein originating from organic matter was decomposed into amino acids via peptides and converted to ammonium and nitrate. There was a strong competition with microorganisms for nitrogen.

In the previous section, we proposed 2 hypotheses for the high nitrogen uptake by upland rice. Hypothesis (1) was ruled out, because the rate of organic nitrogen degradation to amino acids was not essential for the high nitrogen uptake by upland rice. Hypothesis (2) was supported based on ^{15}N-labelled RBS application, amino acid supply test and the analysis of the cultivated soils. We concluded that upland rice preferentially takes up ammonium, amino acids and protein, whereas maize takes up nitrate mainly.

Mechanism of organic nitrogen uptake in crops

Plants take up amino acids to incorporate them with less energy compared to nitrate because amino acids can be directly incorporated into metabolic processes. In fact, sedge grass in tundra soils preferentially took up amino acid nitrogen to inorganic one compared with barley[2]. Also birch growing in moist land took up amino acids rather than nitrate[1]. We estimated that similar phenomena may occur in the rhizosphere of upland rice under upland field conditions when organic materials such as RBS were applied.

There are several reports on the absorption of protein by plants. For example, upland rice and barley also absorbed proteins such as albumin and hemoglobin and grew well in solution culture, while naked barley grew better in RNA solution than in nucleotides derived from the decomposition of RNA[6]. A mechanism of "heterophagy" for direct protein uptake has been reported in upland rice[7]. Hemoglobin molecules were taken up by the cell membrane in upland rice through PL-invagination, then moved to vacuoles to be digested for nitrogen nutrition. This mechanism suggests that direct

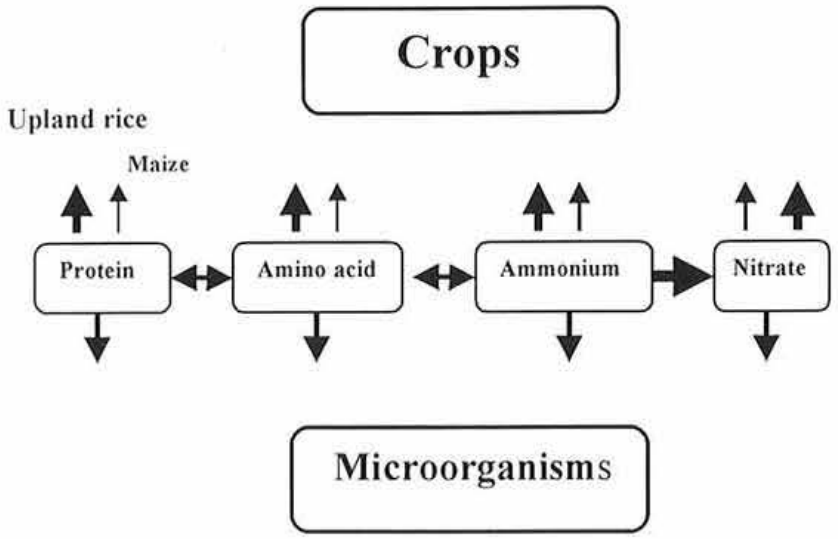

Fig. 8. Scheme of nitrogen mineralization-immobilization cycle in soil and utilization of various nitrogen forms by crops and microorganisms

protein uptake by upland rice under upland field conditions may occur. This mechanism can be attributed to the strong response of upland rice to organic nitrogen, since nitrogen in the protein form would be taken up with less competition for soil nitrogen.

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