



RESEARCH PAPER

# Differential responses of growth and nitrogen uptake to organic nitrogen in four gramineous crops

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## Abstract

The capability to utilize different forms of nitrogen (N) by sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), maize (*Zea mays*), and pearl millet (*Pennisetum glaucum*) was determined in pot experiments. Seedlings were grown for 21 d without N, or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate, rice bran or a mixture of rice bran and straw. No treatment-dependent changes of root length, surface area, and fractal dimension were observed. Shoot growth and N uptake in maize and pearl millet correlated with the inorganic N (ammonium and nitrate) concentration in the soil, suggesting that these species depend upon inorganic N uptake. On the other hand, shoot growth and N uptake patterns in sorghum and rice indicated that these two species could compensate low inorganic N levels in the organic material treatments by taking up organic N (proteins). Analysis of N uptake rates in solution culture experiments confirmed that sorghum and rice roots have higher capabilities to absorb protein N than maize and pearl millet.

Key words: Maize, nitrogen uptake rate, organic nitrogen, *Oryza sativa*, pearl millet, *Pennisetum glaucum*, rice, root system morphology, sorghum, *Sorghum bicolor*, *Zea mays*.

## Introduction

Using organic materials as a nitrogen (N) source for crop production is an important concept of sustainable agriculture (Boddey *et al.*, 1997; Snapp *et al.*, 1998; Cakmak, 2002). Once organic materials are applied to the soil, they will be decomposed by soil microbes, converting proteins into amino acids and, finally, into the inorganic forms of N

(ammonium, and nitrate). Nitrate is generally considered the most readily available N source for plants. However, some plants possess an ability to use other N sources as well, giving them advantages in the competition for available N in their communities (Schmidt and Stewart, 1997).

The low temperatures and acid soil pH commonly found in the arctic and alpine ecosystems inhibit nitrification, leading to ammonium accumulation in the soils (Haynes and Goh, 1978; Klavivko and Keeney, 1987). The plants adapted to such environments depend on the absorption of ammonium rather than nitrate for N acquisition (Atkin, 1996). Furthermore, seasonal changes in the availability of different forms of soil N in the arctic, alpine, and heathland communities confer an advantage to species which flexibly utilize available N sources, including soluble organic compounds such as amino acids (Chapin *et al.*, 1993; Atkin, 1996; Raab *et al.*, 1996; Schmidt and Stewart, 1997). A highly flexible N-acquisition strategy is exemplified by the poikilohydric aquatic plant, *Chamaeigigas intrepidus*, which lives in ephemeral pools with diurnal fluctuations of ammonium and amino acid concentrations in the Namibian desert (Schiller *et al.*, 1998).

The actual pool sizes of organic forms of N (particularly proteins) can be large even in agricultural soils (Mengel, 1996; Matsumoto *et al.*, 2000). Therefore, an ability of crops to take up organic N would seem advantageous for crop productivity. In fact, some agricultural plant species have been shown to absorb organic N. The N uptake of rice (*Oryza sativa*) increased depending on organic N supply rather than on nitrate application (Yamagata and Ae, 1996). Timothy (*Phleum pratense*), buttercup (*Ranunculus acris*), alsike-clover (*Trifolium hybridum*), and red clover (*T. pratense*) were demonstrated to take up glycine (Näsholm *et al.*, 2000). In experiments conducted under sterile

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conditions, carrot (*Daucus carota*) and Chinese cabbage (*Brassica campestris*) absorbed protein-like N compounds without prior mineralization (Matsumoto *et al.*, 2000). Moreover, an uptake of free amino acids through active transport mechanisms has been demonstrated in maize (*Zea mays*; Jones and Darrah, 1994).

Among the cereal crops, the uptake of organic N has been often studied in rice and maize, whereas sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) have received little attention, despite their importance for global food production. Previous results from field trials indicated that sorghum and rice could use soil organic N more efficiently than pearl millet and maize (Okamoto *et al.*, 2003). However, since experiments conducted in the field do not allow the complete control of environmental factors, the present study was performed to investigate the effects of organic N application on growth and N uptake in four gramineous crops (sorghum, rice, maize, and pearl millet) grown under controlled conditions in pots. Furthermore, the capabilities of organic N uptake in the four species were determined in solution culture experiments.

## Materials and methods

### Experiment 1: Growth response to application of different forms of nitrogen in soil culture

**Plant cultivation:** Volcanic ash soil (37.6 g C kg<sup>-1</sup> soil, 2.91 g N kg<sup>-1</sup> soil) was collected from an experimental field at the Japan International Research Center for Agricultural Sciences (Tsukuba, Japan). The field soil was air-dried for 14 d at room temperature and passed through a 2 mm sieve. To reduce the initial N concentration, a 4-fold volume of vermiculite was added to the soil. Nitrogen was applied to the soil at 500 mg kg<sup>-1</sup> soil as ammonium nitrate (AN), rice bran (Bran, C/N ratio=12), a mixture of rice bran and straw (Bran+Straw, C/N ratio=20), or not at all (Control). Phosphorus (P) and potassium (K) were applied as a 5:2 mixture of single superphosphate and fused magnesium phosphate at 750 mg P kg<sup>-1</sup> soil, and as potassium sulphate at 750 mg K kg<sup>-1</sup> soil, respectively. To apply identical rates of P and K in all treatments, the amounts of chemical fertilizer were adjusted according to the P and K contents of the organic materials (details given in Table 1). All materials were mixed with the soil and incubated at 25 °C for 14 d at 60% of the maximum water-holding capacity.

Seeds of sorghum (*Sorghum bicolor* Moench var. Hybrid Sorgo), rice (*Oryza sativa* L. var. Toyohatamochi), maize (*Zea mays* L. var.

Peter No. 610), and pearl millet (*Pennisetum glaucum* L. var. CiVT) were surface-sterilized for 25 min in sodium hypochlorite (available chlorine=5%), and germinated on moistened filter paper (No. 6, Advantec Toyo, Tokyo, Japan) at 25 °C in the dark. Germinated seeds were allowed to grow on vermiculite for 8–14 d without nutrients in a growth chamber (PGW36, Conviron, Manitoba, Canada) at 25 °C air temperature, 70% relative humidity, and a 14 h photoperiod with 410 μmol m<sup>-2</sup> s<sup>-1</sup> photon flux density at plant height.

Seedlings with about 10 cm shoot length (average dry weight of the seedlings: 22 mg in sorghum; 12 mg in rice; 30 mg in maize; and 10 mg in pearl millet) were freed from the seeds with a razor, transplanted into a 1.0 l polyethylene pot filled with 900 ml of incubated soil (one seedling per pot), and grown for 21 d in the growth chamber. Soil moisture content was maintained at 60% of the maximum water-holding capacity by occasional watering with deionized water. During the growth period, measurements of shoot length and chlorophyll concentration in the third leaf counted from the plant apex were performed weekly. Leaf chlorophyll concentration was calculated from values read on a chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan), using calibration curves constructed from chlorophyll (*a+b*) contents determined in ethanol extracts of leaves of each plant species (Osaki *et al.*, 1993). All treatments were replicated three times.

**Plant analysis:** At 21 d after transplanting (DAT), plants were separated into roots and shoots. Root samples were washed in tap water to remove soil particles, rinsed with deionized water, and spread on a flat bed scanner (Scan Jet 4C/T, Hewlett Packard, California, USA). Images of root systems were scanned at 300 dpi resolution. Root length, root surface area, and root fractal dimension were determined using image analysis software (Win/MacRHIZO V3.9e, Régent Instruments, Québec, Canada). Root fractal dimension is an index for the complexity of root branching pattern (Tatsumi *et al.*, 1989; Fitter and Stickland, 1992). Root and shoot samples were dried at 70 °C in an oven for 72 h, weighed, and ground for chemical analysis. Dried samples were digested with H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub>, and N concentrations were determined by the steam distillation method (Distillation Unit Büchi-339, Büchi, Flawil, Switzerland).

**Soil analysis:** Soil samples from pots without plants were collected weekly and samples from pots with plants were collected at the end of experiment for the evaluation of soil N status. Inorganic N was extracted from samples equivalent to 2 g of dry soil with 50 ml of 1.34 M KCl solution by shaking for 30 min at room temperature, and the concentrations of ammonium and nitrate were determined by colorimetric methods (Auto Analyzer-II, Bran+Luebbe, Norderstedt, Germany). Protein N in 10 g of dry soil was extracted with 50 ml of 67 mM phosphate buffer (pH 7.0) by shaking for 60 min at room temperature (Matsumoto *et al.*, 2000), and its concentration was determined by the Bradford method (Bradford, 1976) with bovine serum albumin as a standard (Protein Assay Kit II, Bio-Rad, California, USA).

**Table 1.** Application rates of nitrogen (N), phosphorus (P), and potassium (K) nutrients in pots either without N (Control) or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate (AN), rice bran (Bran), or a mixture of rice bran and straw (Bran+Straw)

Treatment	N (0 or 500 mg kg <sup>-1</sup> soil)			P (750 mg kg <sup>-1</sup> soil)				K (750 mg kg <sup>-1</sup> soil)		
	NH <sub>4</sub> NO <sub>3</sub>	Rice bran <sup>a</sup>	Rice straw <sup>b</sup>	Superphosphate	Fused phosphate	Rice bran	Rice straw	K <sub>2</sub> SO <sub>4</sub>	Rice bran	Rice straw
Control	–	–	–	525	225	–	–	750	–	–
AN	500	–	–	525	225	–	–	750	–	–
Bran	–	500	–	227	97	425	–	449	301	–
Bran+straw	–	425	75	264	113	361	12	316	255	179

<sup>a</sup> Contained 414 mg C g<sup>-1</sup>, 35 mg N g<sup>-1</sup>, 30 mg P g<sup>-1</sup>, and 21 mg K g<sup>-1</sup>.

<sup>b</sup> Contained 361 mg C g<sup>-1</sup>, 6 mg N g<sup>-1</sup>, 1 mg P g<sup>-1</sup>, and 13 mg K g<sup>-1</sup>.

### Experiment 2: Nitrogen uptake rate of plants grown in solution culture

**Plant cultivation:** Germinated seeds of the four species as above were grown on perlite for 9–14 d without nutrients in the growth chamber. Seedlings with about 10 cm shoot length were transferred into a 45 l plastic container filled with 40 l of a standard nutrient solution containing  $\text{NH}_4\text{NO}_3$  (0.5 mM),  $\text{NaH}_2\text{PO}_4$  (0.07 mM), KCl (0.6 mM),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (1 mM),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.8 mM),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (30  $\mu\text{M}$ ),  $\text{H}_3\text{BO}_3$  (50  $\mu\text{M}$ ),  $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$  (9  $\mu\text{M}$ ),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.3  $\mu\text{M}$ ),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.7  $\mu\text{M}$ ), and  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (0.1  $\mu\text{M}$ ). Seedlings were grown in the growth chamber for 7–18 d under constant aeration of the root medium. The pH of the solution was adjusted to 5.5 every day, and the solution was renewed every week.

**Treatment:** Before treatment, seedlings were kept in the standard nutrient solution without N for 7 d. Roots were then washed with deionized water and rinsed with 10  $\text{mg l}^{-1}$  ampicillin solution (pH 5.5). Ampicillin was used to inhibit bacterial growth (Chapin *et al.*, 1993). Sets of 2–5 seedlings (average dry weight of the roots per bottle: 81 mg in sorghum; 136 mg in rice; 139 mg in maize; and 97 mg in pearl millet) were transferred into 50 ml plastic bottles containing 45 ml of aerated treatment solutions. The treatment solutions consisted of the standard nutrient solution without N (Control), or with 0.2 mM N as  $\text{NH}_4\text{NO}_3$  (IN) or organic N extracted from soil as described below (ON). All treatment solutions contained 10  $\text{mg l}^{-1}$  ampicillin. Seedlings were incubated under light conditions for 3 h in the growth chamber. Then, roots were dried at 70 °C in an oven for 72 h, and weighed. The treatment solutions before and after the treatment were filtered through 0.45  $\mu\text{m}$  membrane filters, and the concentrations of inorganic N (ammonium and nitrate) and protein N were determined as described for Experiment 1. Nitrogen uptake rate per unit root ( $\mu\text{mol g}^{-1}$  root DW  $3 \text{ h}^{-1}$ ) was calculated from the changes in the N amount in the solution during the treatment, divided by root dry weight. All treatments were replicated five times.

**Preparation of organic nitrogen in the treatment solution:** The organic N in the ON treatment solution was extracted from the same field soil as used in Experiment 1. A mixture of 3.2 g C as glucose and 0.16 g N as ammonium sulphate (providing a final C/N ratio of 20) was supplied to 1 kg of dry soil to stimulate microbial synthesis of organic N in the soil. Soil was incubated at 25 °C for 14 d at 60% of the maximum water-holding capacity in a 3.0 l plastic bottle. Organic N was extracted from the incubated soil with 2.0 l of 67 mM phosphate buffer (pH 7.0) by shaking for 60 min at room temperature, and then passed through a filter paper (No. 5C, Advantec Toyo). Ions and compounds with low molecular weight (<3500 Da) were eliminated from the soil extract by dialysis using a membrane tube (exclusion size=3500 Da, Spectra/Por CE, Spectrum Laboratories, California, USA) in running tap water at 25 °C for 3 d, then in 10 l of deionized water at 4 °C for 1 d. The dialysed extract was passed through 0.20  $\mu\text{m}$  membrane filter for sterilization. Total N concentration in the soil extract was 0.206 mM including 0.005 mM nitrate N and 0.092 mM protein N after dialysis.

### Statistical analysis

Results were evaluated by analysis of variance (ANOVA) at the  $P < 0.05$  probability level. Fisher's Protected LSD was calculated only when the ANOVA  $F$ -test indicated significant treatment effects at the  $P < 0.05$  level.

## Results

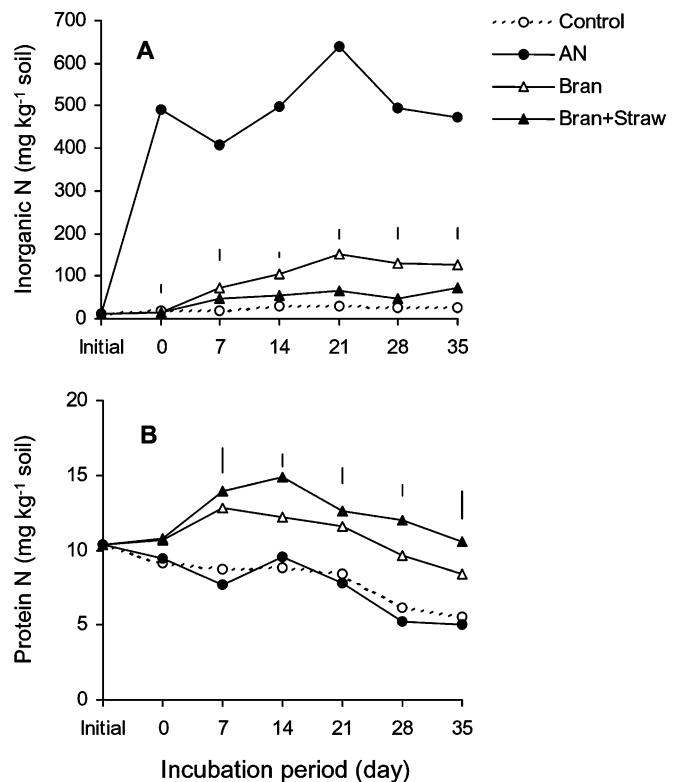
### Experiment 1: Nitrogen concentration in the soil

The inorganic N (ammonium and nitrate) concentration in the soil without plants increased dramatically in the AN

treatment at the beginning of the incubation, and kept the highest level among the treatments (Fig. 1A). Both in the Bran and Bran+Straw treatments, the inorganic N concentration in the soil increased slowly with time, but it was generally higher in the Bran treatment. The inorganic N concentration was lowest in the Control treatment throughout the incubation period.

Soils of the different treatments began to differ significantly in protein N concentration by 1 week after the beginning of incubation (Fig. 1B). Protein N contents increased following the application of organic materials, and were highest in the Bran+Straw treatment. The concentrations were lower in the AN and Control treatments, and decreased continuously during the incubation period. After 2 weeks of incubation, protein N concentrations began to decline at similar rates in all treatments.

At 21 DAT, the inorganic N concentrations in the soil with plants were lower than those in the soil without plants except for the Bran and Bran+Straw treatments of sorghum (Table 2). The amount of inorganic N in the soil grown with sorghum was at the same level as in the soil without plants in the Bran and Bran+Straw treatments. On the other hand, the inorganic N in the soils with maize and pearl millet was



**Fig. 1.** Changes in the concentration of (A) inorganic N (ammonium and nitrate) and (B) protein N in soil without plants, either without N (Control, open circles) or with 500  $\text{mg N kg}^{-1}$  soil applied as ammonium nitrate (AN, closed circles), rice bran (Bran, open triangles), or a mixture of rice bran and straw (Bran+Straw, closed triangles). Data shown are means of three replications with LSD values ( $P < 0.05$ ).

almost depleted in the Bran+Straw treatment as well as in their Control treatment.

### Experiment 1: Growth responses of plants

Significant differences in shoot length and leaf chlorophyll concentration appeared between the treatments after 14 and 7 DAT, respectively, except for sorghum in which such effects were not observed before 21 DAT (Fig. 2). In sorghum and rice, shoot length and leaf chlorophyll concentration were promoted by N application (AN, Bran, and Bran+Straw) irrespective of the N form. Contrarily, shoot length and leaf chlorophyll concentration in maize and pearl millet were significantly increased only in the AN and Bran treatments, whereas the Bran+Straw treatment hardly differed from the Control treatment.

The effects of different forms of applied N on shoot dry weight at 21 DAT were similar to those on shoot length and leaf chlorophyll concentration (Table 3). Sorghum and rice developed similar increases of shoot dry weight in all N treatments. By contrast, shoot dry weights of maize and pearl millet were promoted most strongly in the AN

followed by the Bran treatment, while the effects of the Bran+Straw were insignificant.

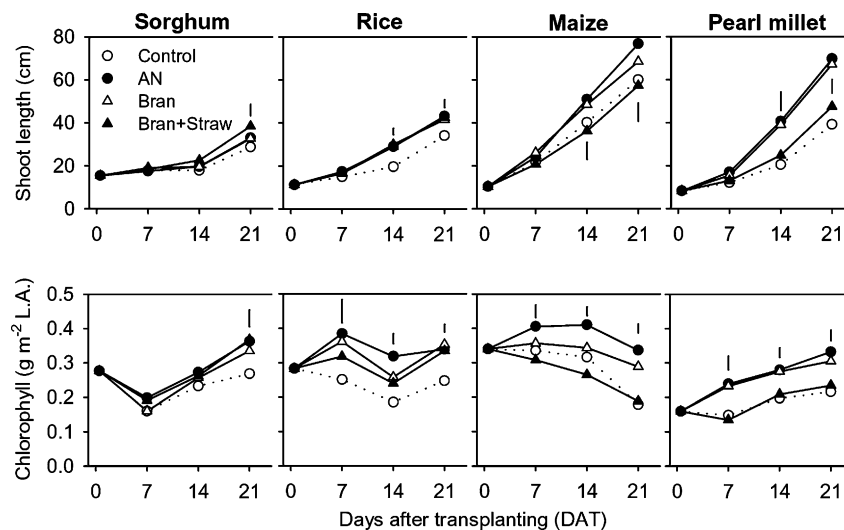
Compared with the shoot, root growth was less affected by the N treatments (Table 3). Sorghum did not show any significant differences between the treatments regarding root dry weight and shoot-to-root ratio. Root dry weight of rice in the AN treatment was similar to the Control treatment, and was slightly increased in the Bran and Bran+Straw treatments. Root dry weights of maize and pearl millet were significantly higher in the AN and Bran treatments than in the Bran+Straw and Control treatments; a similar tendency was observed in the shoot-to-root ratio in pearl millet but not in maize.

### Experiment 1: Root system morphology

In sorghum, total root length, specific root length, surface area, and fractal dimension of the root system hardly responded to applied N (Table 4). The fractal dimension of the rice root system showed a slight increase in the AN and Bran treatments. Maize showed a significant decrease of specific root length and an increase of root surface area in the AN treatment. Root morphological parameters in pearl

**Table 2.** Concentration of inorganic N (ammonium and nitrate) in soil with or without plants at 21 DAT, either without N (Control) or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate (AN), rice bran (Bran), or a mixture of rice bran and straw (Bran+Straw). Data shown are means of three replications. Numbers in parentheses are the differences between the soil with and without plants.

Treatment	Inorganic N (mg kg <sup>-1</sup> soil)				
	Without plants	Sorghum	Rice	Maize	Pearl millet
Control	24	16 (-9)	11 (-14)	15 (-10)	12 (-12)
AN	471	375 (-96)	410 (-61)	361 (-110)	295 (-176)
Bran	126	132 (+6)	96 (-30)	79 (-47)	39 (-87)
Bran+Straw	71	78 (+7)	27 (-44)	14 (-57)	18 (-52)



**Fig. 2.** Changes in shoot length and leaf chlorophyll concentration of the four species grown in pots either without N (Control) or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate (AN), rice bran (Bran), or a mixture of rice bran and straw (Bran+Straw). Data shown are means of three replications with LSD values ( $P < 0.05$ ).

millet were significantly improved (including the decrease of specific root length) in the AN and Bran treatments.

### Experiment 1: Effects of different nitrogen forms on plant nitrogen uptake

The uptake of N in sorghum and rice at 21 DAT was promoted by N application with little differences between

**Table 3.** Plant dry weight and shoot-to-root ratio of the four species at 21 DAT, grown in pots either without N (Control) or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate (AN), rice bran (Bran), or a mixture of rice bran and straw (Bran+Straw)

Data shown are means of three replications.

Plant	Treatment	Dry weight (mg plant <sup>-1</sup> )			Shoot/root ratio (mg mg <sup>-1</sup> )
		Shoot	Root	Total	
Sorghum	Control	95 a <sup>a</sup>	35 ns	130 a	2.7 ns
	AN	162 ab	55	216 ab	3.1
	Bran	138 a	47	184 a	3.0
	Bran+Straw	221 b	70	291 b	3.2
Rice	Control	87 a	34 a	121 a	2.5 a
	AN	148 b	38 a	186 b	3.9 b
	Bran	172 b	51 b	222 b	3.4 c
	Bran+Straw	147 b	41 ab	189 b	3.5 bc
Maize	Control	521 ab	180 a	701 a	2.9 ns
	AN	918 c	343 b	1261 b	2.7
	Bran	784 ac	291 b	1074 b	2.7
	Bran+Straw	375 b	161 a	536 a	2.4
Pearl millet	Control	136 a	39 a	175 a	3.5 a
	AN	828 b	122 b	951 b	6.8 b
	Bran	640 c	139 b	779 c	4.7 c
	Bran+Straw	202 a	57 a	260 a	3.5 a

<sup>a</sup> Different letters within the treatments of each species indicate significant differences at the  $P < 0.05$  level according to the LSD test. ns: not significant.

**Table 4.** Total root length, specific root length, root surface area, and root fractal dimension of the four species at 21 DAT, grown in pots either without N (Control) or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate (AN), rice bran (Bran), or a mixture of rice bran and straw (Bran+Straw)

Data shown are means of three replications.

Plant	Treatment	Root length (m)	Specific root length (m g <sup>-1</sup> )	Root surface area (×10 <sup>-4</sup> m <sup>2</sup> )	Root fractal dimension
Sorghum	Control	4.7 ns <sup>a</sup>	134.5 ns	52.5 ns	1.49 ns
	AN	6.5	121.1	84.6	1.55
	Bran	5.1	120.3	67.9	1.51
	Bran+Straw	7.7	109.7	106.8	1.55
Rice	Control	5.2 ns	152.3 a	58.2 ns	1.48 a
	AN	4.5	119.0 b	61.4	1.53 b
	Bran	5.7	114.1 b	76.1	1.52 b
	Bran+Straw	5.0	121.2 b	63.3	1.48 a
Maize	Control	13.6 ns	75.6 a	209.7 a	1.60 ns
	AN	17.6	51.3 b	328.6 b	1.68
	Bran	15.4	53.6 b	259.9 ab	1.63
	Bran+Straw	14.7	92.1 c	206.3 a	1.60
Pearl millet	Control	4.6 a	119.5 a	55.5 a	1.42 a
	AN	8.5 bc	70.6 b	160.0 b	1.56 b
	Bran	10.6 c	77.6 b	179.6 b	1.57 b
	Bran+Straw	6.3 ab	109.4 a	83.2 a	1.48 c

<sup>a</sup> Different letters within the treatments of each species indicate significant differences at the  $P < 0.05$  level according to the LSD test. ns: not significant.

the forms of applied N (Fig. 3). Nitrogen uptake was highest in the Bran+Straw treatment in sorghum, and in the Bran treatment in rice. By contrast, N uptake was not increased in the Bran+Straw treatment in maize and pearl millet, while the Bran and particularly the AN treatments had dramatic effects in these species (Fig. 3).

### Experiment 2

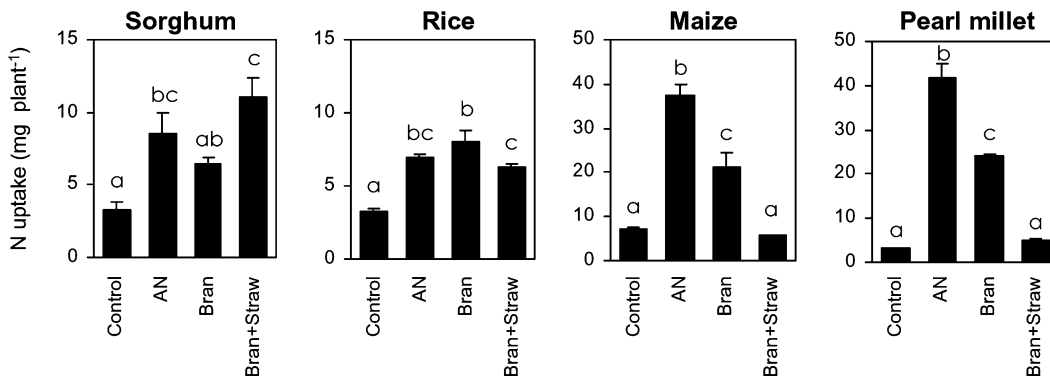
In the Control and IN treatments, an efflux of protein N from roots into the medium was observed in all species (Fig. 4). Maize also exuded substantial amounts of ammonium N in the Control and ON treatments. The rates of inorganic N (ammonium and nitrate) uptake and protein N efflux of pearl millet were highest among the plant species in the IN treatment. The ammonium N in the root medium of the IN treatment was depleted in all species during the treatment period. The nitrate N in the IN treatment solution decreased by 22% in sorghum, 91% in rice, 54% in maize, and 59% in pearl millet from the initial content.

In the ON treatment, the initially contained nitrate N was entirely depleted in all plant species after the treatment. Sorghum and rice showed about 2-fold higher rates of protein N uptake than maize (Fig. 4). Pearl millet seemed incapable of taking up protein N in the ON treatment. The proportion of decreased protein N in the ON treatment was 21% in sorghum, 33% in rice, 18% in maize, and 0% in pearl millet during the treatment period.

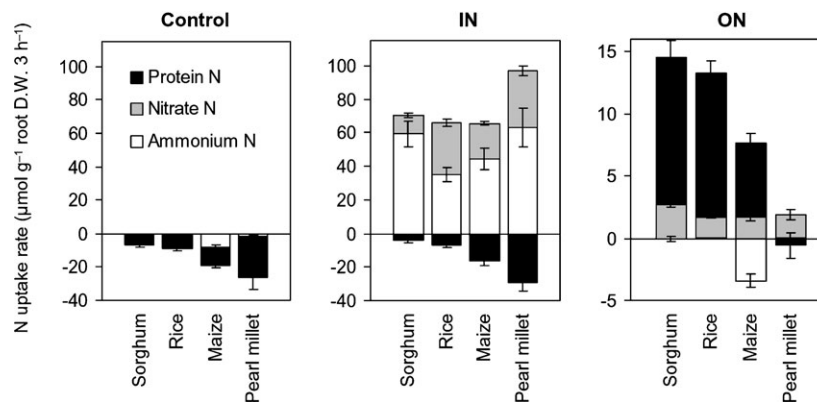
## Discussion

### Experiment 1: Soil nitrogen status

Soil N status varied with the kind of applied N source. Inorganic N (ammonium and nitrate) concentrations in the



**Fig. 3.** Nitrogen uptake of the four species at 21 DAT, grown in pots either without N (Control) or with 500 mg N kg<sup>-1</sup> soil applied as ammonium nitrate (AN), rice bran (Bran), or a mixture of rice bran and straw (Bran+Straw). Data shown are means of three replications with standard error. Different letters indicate significant differences ( $P < 0.05$ ).



**Fig. 4.** Nitrogen uptake rates (ammonium, nitrate, and protein) in the four species in hydroponic culture either without N (Control) or with 0.2 mM N applied as NH<sub>4</sub>NO<sub>3</sub> (IN) or as phosphate-buffer-extractable organic N (ON) in the root medium. Data shown are means of five replications with standard error.

soil without plants increased greatly after the application of inorganic but not organic N sources (Fig. 1A), as also shown in previous studies (Yamagata and Ae, 1996; Matsumoto *et al.*, 2000). Although organic materials generally release inorganic N through decomposition by soil microbes, rice bran was more effective in increasing soil inorganic N than a mixture of rice bran and straw, probably because of higher mineralization rates due to its lower C/N ratio (Qian and Schoenau, 2002).

By comparison with the inorganic N concentration, the protein N concentration in the soil was much lower, even in the organic N treatments. Since the increasing concentration of extraction phosphate buffer effectively resulted in the higher amount of organic N extracted from the soil (Okamoto, 2004), the available amount of soil protein N in each treatment might have been underestimated in the present method using phosphate buffer of low concentration (i.e. 67 mM). Therefore, the present values of soil protein N concentration were used for comparing the treatment effects, but cannot be compared with the values of soil inorganic N concentration.

The protein N concentration in the soil increased in the organic N treatments (Bran and Bran+Straw) whereas it decreased in the AN and Control treatments (Fig. 1B). Higher concentrations of protein N in the Bran+Straw than in the Bran treatment suggested that organic material with a high C/N ratio might promote the microbial resynthesis of proteins in the soil rather than mineralization (Miltner and Zech, 1999).

The four plant species responded differently to N treatments. It is likely that improved physical properties of the soil caused by the addition of organic materials might affect root growth and, consequently, whole plant development. There is ample evidence for positive effects of organic matter on the soil structure, aggregation (Chantigny *et al.*, 1997), porosity (Barzegar *et al.*, 2002), and water-holding capacity (Sonnleitner *et al.*, 2003), all of which can promote root development. In the current study, however, the addition of a 4-fold volume of vermiculite to the field soil and the maintenance of soil moisture at 60% of the maximum water-holding capacity probably minimized differences in the physical properties of the soil between

the treatments. Therefore, the observed effects on plant growth and development were probably caused by the different N sources rather than by the physical properties of the soil.

#### Experiment 1: Responses of maize and pearl millet

In maize and pearl millet, shoot length in the AN and Bran treatments increased with time to exceed that in the Bran+Straw and Control treatments significantly by 14 d after transplanting (Fig. 2). Maize and pearl millet developed the highest dry weights in the AN treatment followed by the Bran treatment (Table 3). Growth of both species was not promoted by N application as a Bran+Straw mixture. Moreover, the low shoot-to-root ratio in the Bran+Straw treatment in both species implied a low N status in the plants (Freijssen and Otten, 1987).

Root growth responses to N treatments were similar to those of shoot growth in maize and pearl millet (Table 4). Root morphological parameters of these species tended to be positively influenced by the application of inorganic N but not organic N, except for root length and root fractal dimension of maize (Table 4). The poor growth of the roots in the Bran+Straw and Control treatments might imply low N availability in the soil (Eghball *et al.*, 1993; Paterson and Sim, 2000).

Leaf chlorophyll concentrations, an indicator of leaf N status (Maranville *et al.*, 2002), responded to the treatments consistently with shoot length in maize and pearl millet (Fig. 2). Consequently, N uptake correlated with plant dry weight, and corresponded to the inorganic N concentration in the soil (AN > Bran > Bran+Straw  $\approx$  Control; Figs 1A, 3). Thus, growth and N uptake of maize and pearl millet seem generally to depend on the availability of soil inorganic N. The fact that the highest K concentrations in maize and pearl millet tissues were found in the AN treatment (data not shown) is in line with this interpretation because nitrate uptake enhances the uptake of K<sup>+</sup> as a counterion (Van Beusichem *et al.*, 1988).

#### Experiment 1: Responses of sorghum and rice

By contrast with maize and pearl millet, the growth responses to N treatments of sorghum and rice did not correspond to the soil inorganic N concentration. Sorghum and rice developed significant increases in shoot length (Fig. 2), leaf chlorophyll concentration (Fig. 2), plant dry weight (Table 3), and N uptake (Fig. 3) in all N treatments, despite large differences in soil inorganic N contents. Since root growth of sorghum and rice hardly responded to the treatments (except for root dry weight, specific root length, and root fractal dimension of rice; Table 4), the significant responses of these species to the treatments might not be due to root development but to N availability.

The similar amounts of N uptake in sorghum and rice between the forms of applied N can possibly be explained by the abundant amounts of soil inorganic N for the two

species with a relatively smaller size of plant than maize and pearl millet. The inorganic N supply, even from the organic materials, might have been enough for sorghum and rice to result in the high N uptake as the plants that were supplied with inorganic N. For example, the residual amounts of soil inorganic N in the Bran+Straw treatment of sorghum and rice were still higher than in the Control treatment at 21 DAT (Table 2), indicating that these species had not yet depleted the soil inorganic N.

There may be another possibility to explain the different response patterns of sorghum and rice to N treatments from those of maize and pearl millet. The significantly increased levels of soil protein N in the Bran and Bran+Straw treatments than in the other two treatments (Fig. 1B) might have contributed to N acquisition of sorghum and rice because plant roots seem able to take up proteins to some extent (McLaren *et al.*, 1960; Nishizawa and Mori, 1977; Abuzinadah *et al.*, 1986).

Nitrogen uptake of sorghum was higher in the Bran+Straw treatment than in the Bran treatment (Fig. 3), and thus correlated with the order of soil protein N concentration (Fig. 1B), while rice showed the opposite response. This might indicate that in the Bran and Bran+Straw treatments, sorghum depended critically on protein N uptake, while rice might use protein N merely as a supplement for the scarce inorganic N. The comparable amounts of residual inorganic N in the soil grown with sorghum to without plants in the Bran and Bran+Straw treatments (Table 2) could imply that sorghum did not take up inorganic N at least in these treatments. Sorghum experiencing the low status of soil inorganic N in the organic N treatments might have switched to utilizing protein N preferentially instead of inorganic N like *C. intrepidus*, which takes up amino acids as well as ammonium depending on the availability in the root medium (Schiller *et al.*, 1998). Such adaptability could be responsible for the observed delay in the appearance of treatment effects on plant growth in sorghum (Fig. 2).

#### Experiment 2: Capability for protein uptake

The capability to absorb inorganic and organic forms of N was compared between the four plant species in hydroponic culture. All species exuded protein N from roots as shown in the Control and IN treatments (Fig. 4), implying that the uptake rate of protein N in the ON treatment included resorption of exuded proteins from the root medium. Pearl millet exhibited the highest rate of inorganic N uptake in the IN treatment, and the lowest rate of organic N absorption in the ON treatment of all the species, confirming its preference for the uptake of inorganic N as suggested by the results from the soil culture experiments.

In the ON treatment, sorghum and rice exhibited higher abilities to absorb protein N from the root medium than maize and pearl millet. Maize seems capable of taking up soluble proteins as well as amino acids (Jones and Darrah,

1994). However, its high rate of ammonium efflux might have offset the effect of organic N uptake in Experiment 1, resulting in the smaller response of N uptake to organic N application. Otherwise, maize roots might lack the ability to liberate the soil organic N compounds from soil minerals by exuding such as organic acids (S Matsumoto and N Ae, unpublished data).

Compared with the rates of inorganic N uptake in the IN treatment, protein N uptake rates in the ON treatment were low in all plant species (Fig. 4). The 4.5  $\mu\text{mol}$  ammonium N in the IN solution and 0.225  $\mu\text{mol}$  nitrate N in the ON solution were immediately depleted while 67–100% of 4.1  $\mu\text{mol}$  protein N remained in the root medium after the absorption period of 3 h. These results indicated that inorganic N was more easily taken up by plant roots than protein N. However, some reports proved that the preferential form of plant N uptake might depend on the dominant form of N in the root medium through most of their growth period. For example, the uptake rate of glycine N was higher in *Eriophorum vaginatum* grown with amino acids than that grown with either ammonium or nitrate as the sole N source (Chapin *et al.*, 1993). *Kobresia myosuroides* grown with glycine took up glycine N at a higher rate than the uptake rate of nitrate N in that grown with nitrate (Raab *et al.*, 1996). In the current study, plants were grown for 7–18 d with inorganic N (0.5 mM  $\text{NH}_4\text{NO}_3$ ) as the sole N source, and therefore might have adapted to the preferential uptake of inorganic N rather than organic N.

## Conclusion

In conclusion, growth and N uptake differed in the four gramineous crop species depending upon the form of N applied. Maize and pearl millet showed increases in growth and N uptake following the application of inorganic N, and appeared unable to use organic forms of N efficiently. Intriguingly, pearl millet seemed to depend entirely on inorganic N while maize showed a limited capability for protein N uptake. Sorghum and rice seemed to prefer inorganic N uptake, but also to possess the high capabilities to take up protein N compared with maize and pearl millet. The differential responses between the plant species in the soil culture experiments to organic N application could be partly due to the difference in plant characteristics of organic N utilization.

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