# Nitrogen uptake and transpiration by plant effects on nitrate leaching from granitic regosol

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**Running title: NO3-N leaching affected by plant growth**

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## ABSTRACT

Field and soil column experiments were conducted to analyze the effects of N uptake and transpiration by corn on nitrate-nitrogen ($NO_3$-N) leaching in a granitic regosol and to evaluate the contribution of plant growth to the reduction of $NO_3$-N leaching. In the field experiment, $NO_3$-N leaching, N uptake by corn and retained N (inorganic N and microbial biomass N) were monitored in conventional planting density (CPD), high planting density (HPD) and no planting (NP) treatments. Nitrogen (N) was applied as $(NH_4)_2SO_4$ at the rate of 300 kg N ha$^{-1}$ and corn (*Zea mays* L.) was sown. In the soil column experiment, 1500 mg N column$^{-1}$ was applied and corn was sown in four treatments; the no plant (NP), 1-, 2- and 4-plant column$^{-1}$. $NO_3$-N leaching, N uptake and transpiration by corn and retained $NO_3$-N in the soil were measured. In the field experiment, cumulative $NO_3$-N leaching in the NP treatment was 208 kg N ha$^{-1}$ from 38 to 49 days after treatment. In the CPD and HPD treatments, $NO_3$-N leaching was reduced to 148 kg N ha$^{-1}$ and 73 kg N ha$^{-1}$, respectively. N uptake by corn and retained N in the soil increased with increasing of planting density. Cumulative $NO_3$-N leaching was negatively correlated with N uptake by corn ($r = -0.940$, $P < 0.01$, $n = 10$). $NO_3$-N leaching decreased as N uptake by corn increased above 60 kg N ha$^{-1}$. In the soil column experiment, cumulative $NO_3$-N leaching was decreasing with increasing of planting density because of increasing of N uptake by corn and the amount of retained $NO_3$-N in soil. The amount of retained $NO_3$-N in soil was positively correlated with transpiration by corn ($r = 0.943$, $P < 0.01$, $n = 12$). We concluded that $NO_3$-N leaching from a granitic regosol during the rainy season could be reduced by the increasing of planting density due to the increase of N uptake by plants and the increase of retained N in soil derived from the increasing of transpiration by plants.

Key words

Nitrate leaching, N uptake, transpiration, corn, granitic regosol, planting density

## INTRODUCTION

To ensure crop yield or as a result of tradition, more N fertilizer than necessary is applied to crops. Excess fertilized N is transformed into nitrate and is easily leached from arable soil and polluting river, marine and ground water resources (Andraski *et al*. 2000; Thomsen *et al*. 1993; van der Ploeg *et al*. 1997). This nitrate-nitrogen leaching ($NO_3$-N leaching) from arable soil is a worldwide concern. If rainfall is high in the period following harvesting of the main crop, as is the case in Europe, the use of cover crops is effective for reducing $NO_3$-N leaching. Cover crops reduce residual N in the soil and also the deep percolation of rainfall, consequently reducing $NO_3$-N leaching (Brandi-Dohrn *et al*. 1997; Logsdon *et al*. 2002; Macdonald *et al*. 2005). It is considered that a decrease in soil N content by plant N uptake and a decrease in deep percolation by transpiration of plants can reduce $NO_3$-N leaching from arable soil.

The Chugoku district of Japan is characterized as having an Asian monsoon climate with heavy rains in the main cropping season. The heavy rain often occurs after the application of fertilizer for the main crop which is in the early growing stage. Therefore, $NO_3$-N leaching occurs easily in soils with high $NO_3$-N content during cropping. $NO_3$-N leaching from arable soil depends on soil texture and is greatest in sandy soil (Bergström & Johansson 1991; Geleta *et al*. 1994; Sogbedji *et al*. 2000). Granitic regosols have a sandy texture with poor physical, chemical and biological characteristics and are widely distributed in the Chugoku district of Japan (Herai *et al*. 2006). $NO_3$-N leaching from arable soil also depends on the rate of N fertilizer application and the soil N content (Decau *et al*. 2004; Trindade *et al*. 1997). Large amounts of $NO_3$-N leaching occur with chemical fertilizer application. To reduce $NO_3$-N leaching from granitic regosol during the June-July rainy season, two types of method are mainly considered. The reduction of $NO_3$-N level in soil by plant N uptake is one method. This may be enhanced by controlling the planting density and timing of sawing or by selection of crop which has a higher rate of N uptake. To reduce $NO_3$-N level in soil, it is also effective to use controlled N release fertilizer (Quiroga-Garza *et al*. 2001), or nitrification inhibitor (Ball-Coelho & Roy 1999). Increasing the amount of retained N (inorganic N and microbial biomass N) temporarily in soil during the rainy season is the other method. This may be controlled by microbial biomass N formation using organic manure or by the decrease in deep percolation of $NO_3$-N in the soil with transpiration by plants. The retained N in the soil is available for plants after the rainy season because $NO_3$-N leaching hardly occurs after the rainy season. Herai *et al*. (2006) reported that $NO_3$-N leaching from granitic regosol was less with compost application than with chemical fertilizer application, and that the increase in microbial biomass N with compost application reduced soil inorganic N and consequently reduced $NO_3$-N leaching.

$NO_3$-N leaching is reduced with compost application in the short-term, but in the long-term compost application increases inorganic N derived from the decomposition of organic N accumulated in the soil, and there is a concern that as much or more $NO_3$-N leaching occurs as with chemical fertilizer application (Maeda *et al*. 2003). In addition, farmers often apply chemical fertilizer, which is easier to use rather than compost. In these situations, it is considered that the effect of plant growth on $NO_3$-N leaching becomes more important. In addition, the increases in N uptake and water use efficiency with the development of root systems (Benjamin *et al*. 1996; Kristensen & Thorup-Kristensen 2004), the increase in microbial biomass with plant growth, and the rate of immobilization of N (Joergensen 2000) and denitrification with increasing microbial biomass (Qian *et al*. 1997) may also contribute to reduce $NO_3$-N leaching. Therefore, it is considered that plant growth, which results in N uptake, transpiration, and development of the root system, is an important factor affecting $NO_3$-N leaching from soil if heavy rain occurs in the main cropping season.

The objectives of this study were: to analyze the effects of N uptake and transpiration by plants on $NO_3$-N leaching from granitic regosol; and to evaluate the contribution of plant growth to the reduction of $NO_3$-N leaching. For this purpose, the field experiment was conducted with N application at the rate of 300 kg N $ha^{-1}$ and 3 levels of planting density of corn. In addition, to analyze the effect of transpiration by plants on $NO_3$-N leaching, the soil column experiment was conducted with 4 levels of planting density.

## MATERIALS AND METHODS

### Field experiment

#### *Experimental site*

The field experiment was carried out at the Hiroshima University experimental field, Hiroshima Prefecture, Japan (34°23’N, 132°42’E). The experimental area falls within the Asian monsoon area and has a mean annual air temperature of 13°C and a mean annual precipitation of approximately 1500 mm (25 years mean) with heavy rainfall in June-July. The weather data for the experimental site was obtained from a weather station located 2.6 km away. The soil was a granitic regosol with a loamy sand texture and containing less than 10% of clay content. Detail of the soil chemical characteristics at initiation of the site was reported by Herai *et al*. (2006).

### *Experimental design*

The experimental plot of chemical fertilizer (CF) - conventional planting density treatment (CPD of 60,000 plants ha$^{-1}$) having 80 cm row spacing and 20 cm plant spacing was initiated in 1996, and has been planted corn as a summer crop. $NO_3$-N leaching, shoot dry matter and N uptake by corn, soil inorganic N and microbial biomass N were monitored in the CF-CPD treatment from May 2003 to September 2005. In the field experiment of 2005, CF-high planting density treatment (HPD of 110,000 plants ha$^{-1}$) having 40 cm row spacing and 20 cm plant spacing, and CF- no planting (NP) treatment were prepared in the same field block. The CF-NP and CF-HPD treatments have been kept free of vegetation since 1996, except for the CF-HPD treatment in 2002. Corn and oats were planted in the CF-HPD treatment in 2002. A plot measuring 5 m × 8 m was used for each treatment with two replicates.

Corn (*Zea mays* L. cv. snowdent127S) was sown on 21 May 2003, 18 May 2004 and 26 May 2005 in the CF-CPD and was sown on 26 May 2005 in the CF-HPD treatment. Nitrogen was applied as $(NH_4)_2SO_4$ at 300 kg N ha$^{-1}$ in each treatment. An available P level of 100 kg P ha$^{-1}$ was maintained with superphosphate and fused magnesium phosphate. Potassium was applied at 200 kg K ha$^{-1}$ as $K_2SO_4$ and dolomite (containing 40% CaO and 15% MgO) was applied at 1 Mg ha$^{-1}$ in each treatment. The chemical characteristics of the soil before fertilizer application in the CF-CPD treatments in 2003 were: pH 6.9 (water), total carbon 4.4g kg$^{-1}$ and total nitrogen 0.4 g kg$^{-1}$. The chemical characteristics of the soil in each treatment in 2005 are presented in Table 1.

Two replicate capillary lysimerters (height × width × length of 500 mm × 300 mm × 600 mm: DAIKI, DIK-6900 COMH-9-30, Kounosu city, Saitama, Japan) were used for each treatment and drainage water was sampled from 80 cm below the soil surface. Drainage samples from the lysimeter were collected 24 h after rainfall (at 28, 36, 43, 48, 54, 62, 83 and 103 days after treatment (DAT) in 2003, at 7, 15, 34, 45, 72, 91, 98 and 107 DAT in 2004, and at 20, 38-41, 44-47, 49, 54, 67, 85, 89, and 109 DAT in 2005). After measuring the drainage volume, drainage samples were stored at 4°C until $NO_3$-N analysis.

Soil was sampled before fertilizer application and subsequently at 0, 9, 15, 30, 60 and 90 (DAT) in each year. The soil samples were collected at a depth of 0-10 cm and sieved (< 2 mm) before being divided into two sub samples; one sample was stored at 4°C for soil $NO_3$-N, $NH_4$-N and microbial biomass N analysis and the other was air-dried for other chemical analysis. Ten plants were collected from each of the two replicates of each treatment and the fresh weight of leaves, stems, and grains was determined at 15, 30, 49, 60 and 90 DAT. Four plants in each treatment were dried at 80°C for at least 48 h before being weighted. The dried samples were finely ground and stored for chemical analysis.

## Soil column experiment

The soil column experiment was conducted from 15 July to 11 August 2005 in greenhouse. The four treatments used were differing planting densities of 0 (no plant), 1, 2 and 4 plants per column (NP, 1-, 2- and 4- plant treatments). All treatments were replicated six times making a total of 24 soil columns and half of the soil columns were sampled before water application on 1 August. The columns were made of cylindrical polyvinyl chloride (PVC) tubes (inner diameter 20cm and length 80cm). The bottom of the PVC tube was capped with a PVC plate which had a hole covered with nylon mesh that was connected to a plug with a tube for collecting drainage samples. The soil column was filled with a sieved (< 2 mm) granitic regosol consisting of 60 cm of subsoil and 15 cm of topsoil. The bulk density of the soil used in the soil column experiment was similar to that of the field soil (1.3 g $cm^{-3}$).

On 15 July, phosphorus and potassium were applied as superphosphate at 200 kg P $ha^{-1}$, as $K_2SO_4$ at 200 kg K $ha^{-1}$ and dolomite was applied at 500 kg $ha^{-1}$ for the 15 cm topsoil in each treatment to adjust soil pH=6.0. Nitrogen was applied to the soil surface using $Ca(NO_3)_2 4H_2O$ at 1500 mg N $column^{-1}$ (corresponding to 476 kg N $ha^{-1}$) to avoid the influence by nitrification of ammonium sulfate. After N application, corn was sown in the 1-, 2- and 4-plant treatments. From 31 July to 9 August, deionized water corresponding to 40 mm of precipitation was applied every day to the soil surface in each soil column for 10 days. The total amount of water applied to the soil column (400 mm) was equal to the average amount of precipitation received during the rainy season in this area. The evapotranspiration from the soil column was calculated by measuring the weight of the soil column every day before water was applied. The weight of plant during water application period was estimated using a growth curve and the weight of plant at the beginning and end of the water application period, then subtracted from the weight of the soil column. The difference in evapotranspiration between the NP treatment and the 1-, 2- and 4-plant treatments were assumed to be transpiration by plant. Drainage samples were collected 24 h after water was applied and stored at 4°C until $NO_3$-N analysis. On 1 and 11 August, shoots were cut at the soil surface and roots and soils were sampled from the topsoil and every 10 cm of the subsoil in each soil column. Roots were sieved from the soil and washed with deionized water. The fresh weight of shoots and roots were measured and dried at 80°C for at least 48 h before being weighted. The dried samples were finely ground and stored for chemical analysis. Soil samples were stored at 4°C until $NO_3$-N analysis.

### Chemical analysis

Drainage samples from the lysimeter and soil column were filtered through a 0.2 μm membrane filter (Dismic-13cp, Toyo Roshi, Tokyo, Japan) and the $NO_3$-N concentration of the samples was determined by ion chromatography. The system was composed of an electric conductivity detector (CM-8000, TOSOH Corporation, Tokyo, Japan) and a thermostatic box (CO-8020, TOSOH Corporation) for the separation column (TSKgel IC-Anion-PW, TOSOH Corporation). The amount of $NO_3$-N leached was determined by multiplying the drainage volume by the $NO_3$-N concentration in the drainage samples.

For soil $NH_4$-N analysis, moist soil containing 10 g dry soil was extracted with 100 ml of 2 M KCl solution for 1 h. The extracts were filtered using ADVANTEC No.5C filter paper (Toyo Roshi) and ammonium concentration was determined by the indophenol blue method with a spectrophotometer (Shimadzu, UVmini-1240, Kyoto, Japan). For soil $NO_3$-N analysis, moist soil containing 10 g dry soil was extracted with 100 ml of deionized water for 1h. The extracts were filtered using ADVATEC No.5C filter paper and $NO_3$-N concentration was determined by ion chromatography. The amount of microbial biomass C and N in soil were determined by the chloroform fumigation extraction method (Brookes *et al*. 1985), and the amount of total organic C and total N dissolved in 0.5 M $K_2SO_4$ extracts were determined using an organic C and total N analyzer (Shimadzu, TOC-V CSN TNM-1, Kyoto, Japan). Microbial biomass C ($B_C$) and N ($B_N$) were calculated from the relationship $B_C = 2.22 \times E_C$ and $B_N = 2.22 \times E_N$, (Chowdhury *et al*. 1999) where $E_C$ and $E_N$ correspond to the amount of organic C and total N extracted from the fumigated samples minus the amount in the non-fumigated samples, respectively. Microbial biomass C and N in 0–10 cm soil were determined by multiplying the bulk density (1.31 g $cm^{-3}$). Total C and N in plant and soil samples were determined by the combustion method with a CN analyzer (Yanaco, MT-700, Kyoto, Japan).

### Statistical analysis

Statistical analyses were carried out using the statistical package SPSS for Windows (version 13.0) with the level of significance ($P$ value) set at $P < 0.01$. Means and standard deviation for each treatment were calculated.

## RESULTS

### Field experiment

#### *Drainage volume, $NO_3$-N concentration and $NO_3$-N leaching*

Cumulative $NO_3$-N leaching and shoot dry matter in the CF-CPD treatment from May 2003 to September 2005 is presented in Fig.1. Cumulative $NO_3$-N leaching in the CF-CPD treatment showed a similar trend each year and increased markedly during the period of about 30 to 60 DAT. The total amount of rainfall during the corn cropping season was 842 mm (3 years average).

In 2005, heavy rainfall was received at 36-48, 81 and 103 DAT (Fig. 2A). The amount of rainfall at 36-48 DAT was 308 mm and particularly heavy rainfall of 213 mm was received at 36-39 DAT including more than 70 mm day$^{-1}$ at 37 and 38 DAT and more than 100 mm day$^{-1}$ of rainfall was observed at 81 and 103 DAT. The drainage volume markedly increased from 38 to 41 DAT and small amounts of drainage were observed at 44-49, 85-89 and 109 DAT (Fig. 2B). The drainage volume was a little lower in the CF-HPD treatment than the CF-NP and CF-CPD treatments but the differences were not statistically significant.

The $NO_3$-N concentration in the drainage water during the period of 38-41 DAT was lowest in the CF-HPD treatment, followed by the CF-CPD treatment and the CF-NP treatment (Fig. 3A). The $NO_3$-N concentration in the drainage water was a little higher in the CF-HPD treatment than the CF-CPD treatments at 44-49 DAT but the difference was not statistically significant. The $NO_3$-N concentration in the drainage water remained above 50 mg N L$^{-1}$ in the CF-NP treatment after 49 DAT, while being very low in the CF-CPD and CF-HPD treatments.

$NO_3$-N leaching markedly increased from 38 to 41 DAT and little leaching was observed at 44-49, 85-89, and 109 DAT in all treatments (Fig. 3B). Cumulative $NO_3$-N leaching until 49 DAT in the CF-NP, CF-CPD and CF-HPD treatments was 208, 148 and 73 kg N ha$^{-1}$, respectively and $NO_3$-N leaching decreased with increasing planting density. Total $NO_3$-N leaching until 109 DAT in the CF-NP, CF-CPD and CF-HPD treatments was 298, 149 and 80 kg N ha$^{-1}$, respectively. The ratio of cumulative $NO_3$-N leaching at 49 DAT to the total amount at 109 DAT in the CF-NP, CF-CPD and CF-HPD treatments was 70, 99 and 91 %, respectively.

### *Shoot dry matter and N uptake*

Shoot dry matter was 2-4 Mg ha$^{-1}$ at 49 DAT and reached 10-14 Mg ha$^{-1}$ at harvest in the CF-CPD treatment from 2003 to 2005 (Fig. 1) Shoot dry matter was lower in 2003 as compared to in 2004 and 2005. In 2005, shoot dry matter at 30 DAT was 0.4 Mg ha$^{-1}$ in the CF-CPD and 1.2 Mg ha$^{-1}$ in the CF-HPD treatment (Table 2). After 49 DAT, shoot dry matter was 1.4-1.7 times higher in the CF-HPD treatment than in the CF-CPD treatment. Shoot N uptake was 12 kg N ha$^{-1}$ in the CF-CPD and 40 kg N ha$^{-1}$ in the CF-HPD treatment at 30 DAT and this increased to 55 kg N ha$^{-1}$ and 103 kg N ha$^{-1}$, respectively at 49 DAT (Table 2). Shoot N uptake was 1.2-3.3 times higher in the CF-HPD treatment than in the CF-CPD treatment during the cropping season.

### *Inorganic N in the surface soil and soil microbial biomass N*

Inorganic N in the surface soil (0-10 cm) was similar in all treatments (Fig. 4) and was high (about 300 kg N ha$^{-1}$) up to 15 DAT, then decreased to 100 kg N ha$^{-1}$ at 30 DAT and became very low after 60 DAT.

Soil microbial biomass N in the CF-CPD treatment increased slightly up to 30 kg N ha$^{-1}$ at 60 DAT and then remained constant (Table 3). Microbial biomass N in the CF-NP treatment was 14 kg N ha$^{-1}$ at 15 DAT and slightly increased during the cropping period. The amount of microbial biomass N in the CF-CPD treatment was about 6-14 kg N ha$^{-1}$ higher than in the CF-NP treatment.

## Soil column experiment

*$NO_3$-N leaching*

$NO_3$-N leaching from the soil column increased from 3 to 7 DAT (timing of water application) and was then negligible after 7 DAT (Fig. 5). Total amounts of $NO_3$-N leaching until the end of the water application period were 416, 427, 403 and 374 kg N $ha^{-1}$ in the NP, 1-, 2- and 4-plant treatments with the 4-plant treatment being significantly less than the NP treatment.

*Drainage and evapotranspiration*

The drainage volume from the soil column decreased with increasing planting density. The total drainage volumes during the 10 days water application period were 331, 307, 281 and 266 mm in the NP, 1-, 2- and 4-plant treatments, respectively. The drainage volume decreased by 24, 50 and 65 mm in the 1-, 2- and 4-plant treatments compared to the NP treatment (Fig. 6A). The cumulative evapotranspiration from the soil column during the water application period increased from 61 to 118 mm with increasing planting density (Fig. 6B). Assuming that the differences in evapotranspiration between the NP treatment and the 1-, 2- and 4-plant treatments were due to transpiration by corn, the transpiration by corn was estimated as 19 -57 mm for 10 days.

*$NO_3$-N concentration in the drainage water*

The $NO_3$-N concentration in the drainage water was the highest at 4 DAT in all treatments and increased up to 600 mg N $L^{-1}$ (Fig. 7). In the 1-, 2- and 4-plant treatments, $NO_3$-N concentrations were lower at 3 DAT and higher at 5-6 DAT than the NP treatment.

*Dry matter and N uptake*

Dry matter and N uptake of shoots and roots at the beginning and end of the water application period are presented in Table 4. Shoot dry matter was 0.1-0.5 Mg $ha^{-1}$ at the beginning of the water application period and increased to 0.8-2.8 Mg $ha^{-1}$ at the end. Root dry matter was 0.04 -0.2 Mg $ha^{-1}$ at the beginning of the water application period and increased to 0.4-1.4 Mg $ha^{-1}$ at the end. Root dry matter was 40-50 % of shoot dry matter. Shoot N uptake at the beginning of the water application period was 2.2-20.8 kg N $ha^{-1}$ and increased to 9.7 -25.1 kg N $ha^{-1}$ at the end. Shoot N uptake corresponded to 0.5-4 and 2-5 % of applied N at the beginning and end of the water application period, respectively. Root N uptake at the end of the water application period was 2.9-9.6 kg N $ha^{-1}$ corresponding to 30-39 % of shoot N uptake.

## DISCUSSION

### *The relationships between $NO_3$-N leaching, retained N in soil and N uptake by corn*

In the field experiment, a large amount of $NO_3$-N leaching was observed after more than 300 mm of heavy rainfall at 36-48 DAT, including more than 70 mm $day^{-1}$ for two consecutive days (Figs 2A, 3B) in 2005. The drainage volume was not significantly different among the three treatments (Fig. 2B) but $NO_3$-N concentration in the drainage water significantly decreased in the CF-CPD and CF-HPD treatments compared to the CF-NP treatment at 36-48 DAT (Fig. 3A). In addition, the transpiration by corn would be low on a rainy day. The effect of the transpiration by corn on drainage volume was probably negligible under heavy rainfall conditions of more than 100 mm over 1-2 days. Therefore, the main reason for the decrease in $NO_3$-N leaching in the CF-CPD and CF-HPD treatments was the decrease in $NO_3$-N concentration in the drainage water.

$NO_3$-N concentration in the drainage water during the rainy season was closely-linked to soil $NO_3$-N contents in the 0-30 cm soil profile (Roth & Fox 1990) or in the 0-120 cm soil profile (Jemison & Fox 1994) under the field condition. In our experiment, inorganic N in surface soil was measured. However, most of $(NH_4)_2SO_4$ applied to the soil was converted into $NO_3$-N by nitrification in the surface soil and $NO_3$-N easily moved to the subsoil with rainfall percolation, and inorganic N in surface soil was very low after 30 DAT (Fig. 4). Consequently, the relationship between $NO_3$-N leaching and inorganic N in surface soil was not clear. Therefore, the retained N in the soil from 0 to 80 cm was estimated from the rate of N fertilizer application, plant N uptake and $NO_3$-N leaching. In this experiment, total N and inorganic N in the soil were very low before fertilizer application (Table 1) and the amount of mineralized N from accumulated organic matter in the CF-CPD treatment was less than 1.5 kg N ha$^{-1}$ for 50 days (data not shown). Hence, the N applied in the preceding cropping season and N mineralized from accumulated organic matter had negligible influence on $NO_3$-N leaching. In addition, there was minimal rainfall before the $NO_3$-N leaching increase at 38-49 DAT, possibly resulting in very small N loss from the denitrification, probably less than 5 kg N ha$^{-1}$ (Nishio *et al.* 2002). Therefore, it could be assumed that the retained N in the soil was almost the same as the difference between N applied in fertilizer and the N lost through plant N uptake and $NO_3$-N leaching. Root N uptake (data not shown) was 10-20% of shoot N uptake which was calculated using the shoot to root ratio and root N concentration obtained from the field experiment. The whole plant N uptake (the sum of shoot and root N uptake), retained N in the soil (calculated as described above) and cumulative $NO_3$-N leaching from the soil in each treatment are presented in Fig. 8A, B and C. At 49 DAT, the amount of retained N in the soil tended to increase with increasing of planting density, and was 92, 93 and 115 kg N ha$^{-1}$ in the soil in the CF-NP, CF-CPD and CF-HPD treatments, respectively. When 300 kg N ha$^{-1}$ of fertilizer was applied, about 90 kg N ha$^{-1}$ of $NO_3$-N was retained in the soil temporarily, therefore about 210 kg N ha$^{-1}$ of $NO_3$-N was available to be leached by rainfall or N uptake by corn during the June-July rainy season. In the CF-NP treatment without N uptake by corn, 208 kg N ha$^{-1}$ of $NO_3$-N was leached at 49 DAT, while in the CF-CPD and CF-HPD treatments, N uptake by corn increased to 59 and 112 kg N ha$^{-1}$, respectively. In addition, the retained N in the soil increased and $NO_3$-N leaching decreased. The relationship between cumulative $NO_3$-N leaching and shoot N uptake during the June-July rainy season from 2003 to 2005 is presented in Fig. 9. The data in this relationship were from measurements at 49 DAT in each year. Cumulative $NO_3$-N leaching and shoot N uptake showed a high negative correlation ($r = -0.940$, $P < 0.01$, $n = 10$). This correlation indicated that increased N uptake by corn during the rainy season was directly reflected in decreased $NO_3$-N leaching. In the CF-NP treatment, the amount of 62 kg N ha$^{-1}$ was leached from the retained N in the soil from 49 to 90 DAT. However, in the CF-CPD and CF-HPD treatments, $NO_3$-N leaching hardly occurred because N uptake by corn increased to 133 and 162 kg N ha$^{-1}$, respectively. This indicated that N uptake by corn after the rainy season was also facilitated to reduce $NO_3$-N leaching.

### *The relationships between $NO_3$-N leaching, retained $NO_3$-N in soil and transpiration by corn*

The effect of transpiration by corn on drainage volume was observed in the soil column experiment with the rate of water application of 40 mm $day^{-1}$ for 10 days. Cumulative $NO_3$-N leaching, the amount of retained $NO_3$-N in the soil and N uptake by corn at the end of water application period in each treatment are presented in Fig. 10. N uptake by corn and the amount of retained $NO_3$-N in the soil increased and $NO_3$-N leaching decreased with increasing planting density. The relationship between the amount of retained $NO_3$-N in the soil at the end of water application and cumulative transpiration by corn for 10 days of water application period is presented in Fig. 11. The amount of retained $NO_3$-N in the soil and cumulative transpiration by corn showed a high positive correlation ($r = 0.943$, $P < 0.01$, $n = 12$) and the amount of retained $NO_3$-N in the soil increased by 5-20 kg N $ha^{-1}$ (corresponding to 1-5 % of $NO_3$-N leaching from the NP treatment; 416 kg N $ha^{-1}$) with 40-60 mm (corresponding to 10-15 % of the total amount of precipitation for 10 days; 400 mm) of transpiration by corn. The increase of $NO_3$-N concentration in the drainage water of the plant treatments tended to delay compared to the NP treatment (Fig.7). This showed that the increase of transpiration by corn led to delay the downward movement of $NO_3$-N in the soil and consequently increased the amount of retained $NO_3$-N in the soil.

In the field experiment, the transpiration by corn during the rainy season was probably negligible because of heavy rain. The retained N in the field soil could be increased if the transpiration rate of corn was high as in the soil column experiment. $NO_3$-N leaching hardly occurred after the rainy season under the field condition because of little amount of rainfall and plant uptake of retained N in the soil. Therefore, it was considered that the increase of retained N in the soil derived from the increasing of transpiration by corn in the field was also facilitated to reduce $NO_3$-N leaching during the rainy season.

In this study, it can be concluded that $NO_3$-N leaching from a granitic regosol during the rainy season could be reduced by the increasing of planting density due to the increase of N uptake by plants and the increase of retained N in soil derived from the increasing of transpiration by plants.

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## Figure legends

Figure 1 Cumulative $NO_3$-N leaching and shoot dry matter in the chemical fertilizer-conventional planting density treatment in the field experiment from May 2003 to September 2005.

Figure 2 (A) Precipitation and (B) Cumulative drainage volume from capillary lysimeter in the field experiment (2005). CF-NP, chemical fertilizer-no planting; CF-CPD, chemical fertilizer-conventional planting density; CF-HPD, chemical fertilizer-high planting density. Error bars represent standard deviation.

Figure 3 Effect of planting density on (A) $NO_3$-N concentration in the drainage water and (B) cumulative $NO_3$-N leaching from capillary lysimeter in the field experiment (2005). CF-NP, chemical fertilizer-no planting; CF-CPD, chemical fertilizer-conventional planting density; CF-HPD, chemical fertilizer-high planting density. Error bars represent standard deviation.

Figure 4 Effect of planting density on the inorganic N in the surface soil (0-10 cm) in the field experiment (2005). CF-NP, chemical fertilizer-no planting; CF-CPD, chemical fertilizer-conventional planting density; CF-HPD, chemical fertilizer-high planting density. Error bars represent standard deviation.

Figure 5 Effect of planting density on $NO_3$-N leaching in the soil column experiment. NP, no plant; 1-plant, 1 plant soil column$^{-1}$; 2-plant, 2plants soil column$^{-1}$; 4-plant, 4 plants soil column$^{-1}$. Error bars represent standard deviation

Figure 6 Effect of planting density on (A) cumulative drainage volume and (B) cumulative evapotranspiration in the soil column experiment. NP, no plant; 1-plant, 1 plant soil column$^{-1}$; 2-plant, 2plants soil column$^{-1}$; 4-plant, 4 plants soil column$^{-1}$. Error bars represent standard deviation.

Figure 7 Effect of planting density on $NO_3$-N concentration in the drainage water in the soil column experiment. NP, no plant; 1-plant, 1 plant soil column$^{-1}$; 2-plant, 2plants soil column$^{-1}$; 4-plant, 4 plants soil column$^{-1}$. Error bars represent standard deviation.

Figure 8 Cumulative $NO_3$-N leaching, whole plant N uptake and the retained N in the soil (A) in the chemical fertilizer-no planting, (B) in the chemical fertilizer-conventional planting density and (C) in the chemical fertilizer-high planting density treatment in the field experiment (2005).

Figure 9 Relationship between cumulative $NO_3$-N leaching and shoot N uptake in the field experiment during the rainy season from 2003 to 2005. The data were from measurements at 49 DAT in each year. ** $P < 0.01$.

Figure 10 Effect of planting density on cumulative $NO_3$-N leaching, plant N uptake and the amount of retained $NO_3$-N in the soil at the end of water application in the soil column experiment. Error bars represent standard deviation.

Figure 11 Relationship between the amount of retained $NO_3$-N in the soil at the end of water application and cumulative transpiration by corn for 10 days of water application period in the soil column experiment. ** $P < 0.01$.

Table 1 Chemical characteristics of the soil before fertilizer application in the field experiment (2005).

| | pH | | Total (g kg$^{-1}$) | | Inorganic N (mg N kg $^{-1}$) | | Olsen-P |
|---|---|---|---|---|---|---|---|
| | $H_2O$ | KCl | C | N | $NH_4$-N | $NO_3$-N | (mg P kg$^{-1}$) |
| CF-NP | 7.7 | 6.0 | 2.5 | 0.3 | 1.9 | 0.0 | 24.8 |
| CF-CPD | 6.8 | 6.3 | 5.0 | 0.5 | 1.8 | 5.5 | 46.8 |
| CF-HPD | 7.9 | 5.6 | 2.7 | 0.3 | 2.4 | 0.0 | 26.1 |

Values are mean (n = 3)

Table 2 Shoot dry matter and shoot N uptake by corn in the field experiment (2005).

| | | 15DAT | 30DAT | 49DAT | 60DAT | 90DAT |
|---|---|---|---|---|---|---|
| Soot dry matter (Mg ha$^{-1}$) | CF-CPD | 0.02 ±0.0 | 0.4 ±0.1 | 3.5 ±0.2 | 5.7 ±0.5 | 14.4 ±0.8 |
| | CF-HPD | 0.04 ±0.0 | 1.2 ±0.3 | 5.9 ±0.4 | 9.1 ±1.4 | 20.7 ±2.9 |
| Shoot N uptake (kg N ha$^{-1}$) | CF-CPD | 0.9 ±0.2 | 12.3 ±1.4 | 54.6 ±0.4 | 59.9 ±6.3 | 111.6 ±7.6 |
| | CF-HPD | 1.6 ±0.3 | 40.4 ±9.4 | 103.2 ±16.6 | 97.8 ±2.1 | 130.7 ±21.8 |

Values are mean ± standard deviation (n = 3).

Table 3 Microbial biomass N in the field experiment (2005).

| | | 0DAT | 15DAT | 30DAT | 60DAT | 90DAT |
|---|---|---|---|---|---|---|
| Microbial biomass N (kg N ha$^{-1}$) | CF-CPD | 15.2 ±1.8 | 19.9 ±1.0 | 22.1 ±0.3 | 30.2 ±4.4 | 27.9 ±1.4 |
| | CF-NP | 15.2 ±1.8 | 14.1 ±2.7 | 15.4 ±1.7 | 16.3 ±2.6 | 22.2 ±1.3 |

Values are mean ± standard deviation (n = 3).

Table 4 Dry matter and N uptake at the beginning and the end of the water application period in the soil column experiment.

| | Dry matter (Mg ha$^{-1}$) | | | | N uptake (kg N ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Shoot | | Root | | Shoot | | Root | |
| | Beginning | End | Beginning | End | Beginning | End | Beginning | End |
| 1-plant | 0.1 ± 0.0 | 0.8 ± 0.1 | 0.0 ± 0.0 | 0.4 ± 0.1 | 2.2 ± 0.3† | 9.7 ± 0.5 | n.d. | 2.9 ± 0.5 |
| 2-plant | 0.2 ± 0.0 | 1.8 ± 0.3 | 0.1 ± 0.0 | 0.9 ± 0.1 | 6.0 ± 0.3† | 17.0 ± 2.2 | n.d. | 6.6 ± 0.2 |
| 4-plant | 0.5 ± 0.0 | 2.8 ± 0.1 | 0.2 ± 0.0 | 1.4 ± 0.0 | 20.8 ± 2.6 | 25.1 ± 2.4 | 4.1 ± 0.6 | 9.6 ± 0.3 |

Values are mean ± standard deviation (n = 3).

†Values including root N uptake.

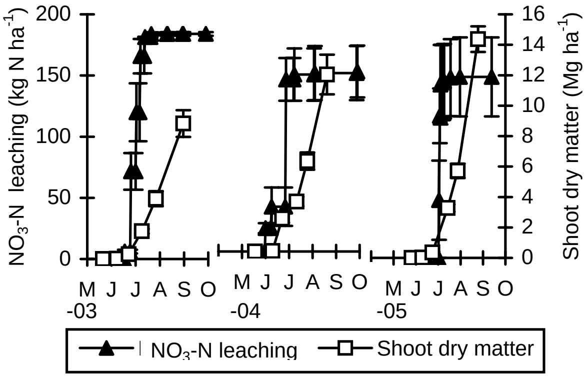

200
150
100
50
0
$NO_3$-N leaching (kg N $ha^{-1}$)
16
14
12
10
8
6
4
2
0
Shoot dry matter (Mg $ha^{-1}$)
M J J A S O
-03
M J J A S O
-04
M J J A S O
-05
$NO_3$-N leaching
Shoot dry matter

**Fig. 1**

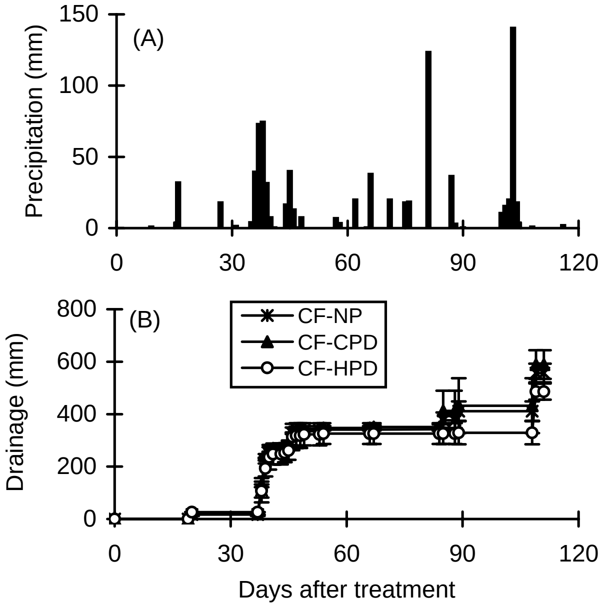

150
(A)
100
50
0
Precipitation (mm)
0
30
60
90
120
800
(B)
600
400
200
0
Drainage (mm)
CF-NP
CF-CPD
CF-HPD
0
30
60
90
120
Days after treatment

**Fig. 2**

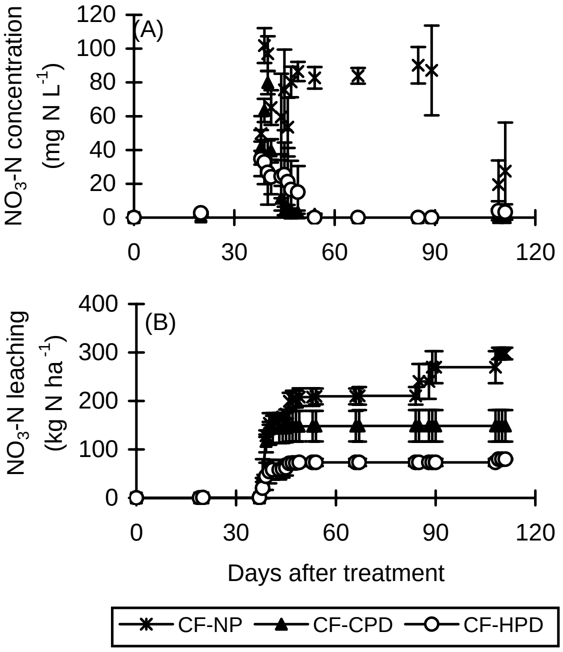
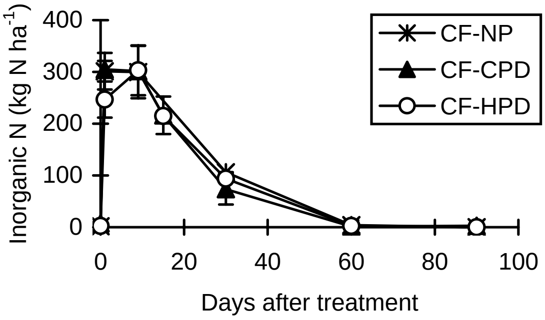

(A)
NO3-N concentration (mg N L-1)
120
100
80
60
40
20
0
0
30
60
90
120
(B)
NO3-N leaching (kg N ha-1)
400
300
200
100
0
0
30
60
90
120
Days after treatment
CF-NP
CF-CPD
CF-HPD

Fig. 3



Inorganic N (kg N ha$^{-1}$)
400
300
200
100
0
0
20
40
60
80
100
Days after treatment
CF-NP
CF-CPD
CF-HPD

**Fig. 4**

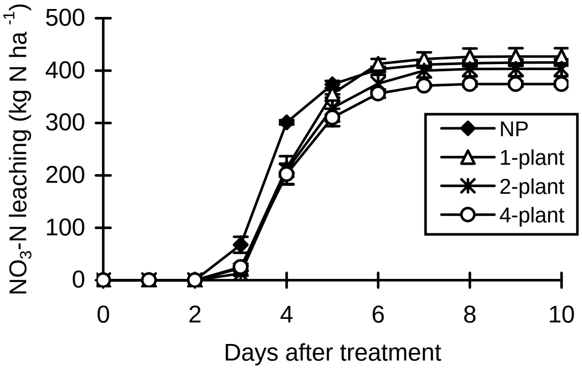
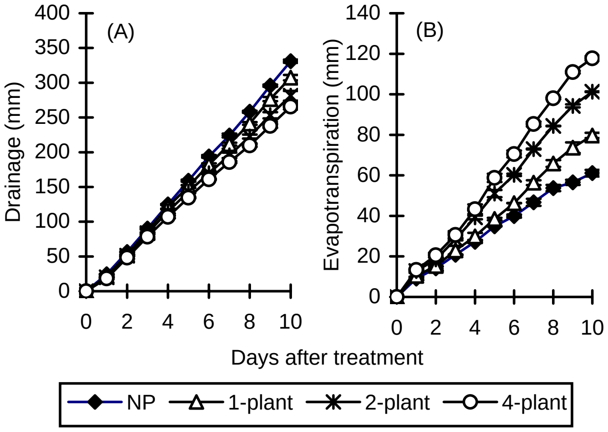

NO$_3$-N leaching (kg N ha$^{-1}$)
500
400
300
200
100
0
0
2
4
6
8
10
Days after treatment
NP
1-plant
2-plant
4-plant

**Fig. 5**



400
350
300
250
200
150
100
50
0
(A)
Drainage (mm)
0
2
4
6
8
10
140
120
100
80
60
40
20
0
(B)
Evapotranspiration (mm)
0
2
4
6
8
10
Days after treatment
NP
1-plant
2-plant
4-plant

**Fig. 6**

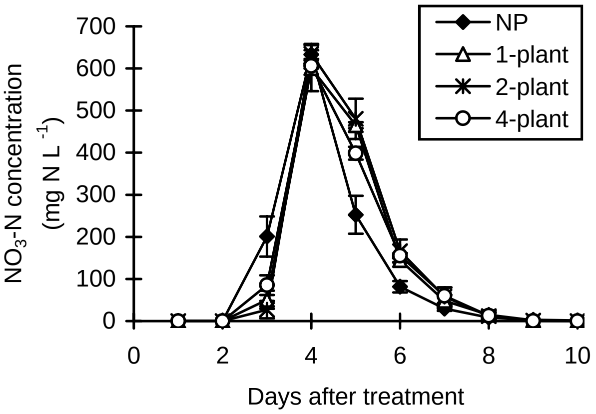

NP
1-plant
2-plant
4-plant
NO$_3$-N concentration (mg N L$^{-1}$)
0
100
200
300
400
500
600
700
0
2
4
6
8
10
Days after treatment

**Fig. 7**

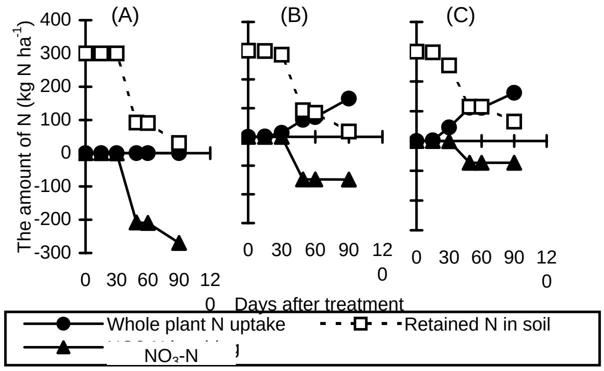

(A)
(B)
(C)
The amount of N (kg N ha$^{-1}$)
400
300
200
100
0
-100
-200
-300
0 30 60 90 120
Days after treatment
Whole plant N uptake
Retained N in soil
NO$_3$-N

Fig. 8

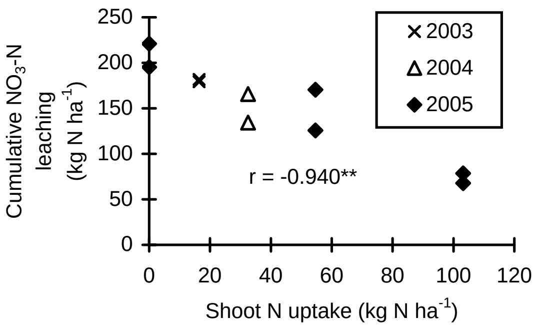

Cumulative NO3-N leaching (kg N ha-1)
250
200
150
100
50
0
0
20
40
60
80
100
120
Shoot N uptake (kg N ha-1)
2003
2004
2005
r = -0.940**

**Fig. 9**

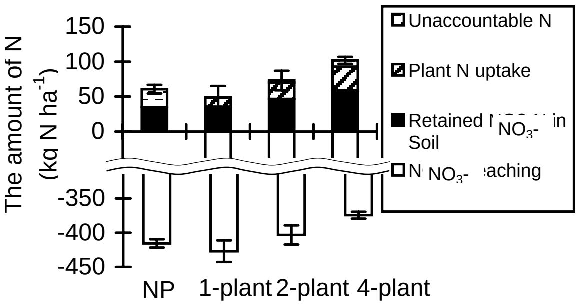

The amount of N
(kg N ha$^{-1}$)
150
100
50
0
-350
-400
-450
NP
1-plant
2-plant
4-plant
Unaccountable N
Plant N uptake
Retained $NO_3$- in
Soil
N $NO_3$- eaching

**Fig. 10**

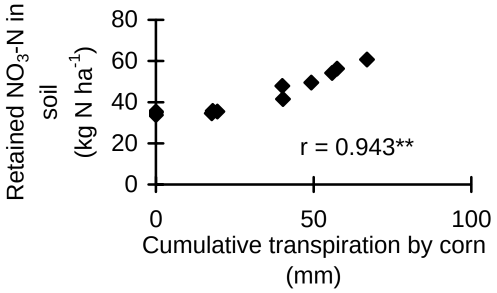

Retained $NO_3$-N in soil (kg N $ha^{-1}$)
80
60
40
20
0
r = 0.943**
0
50
100
Cumulative transpiration by corn (mm)

**Fig. 11**