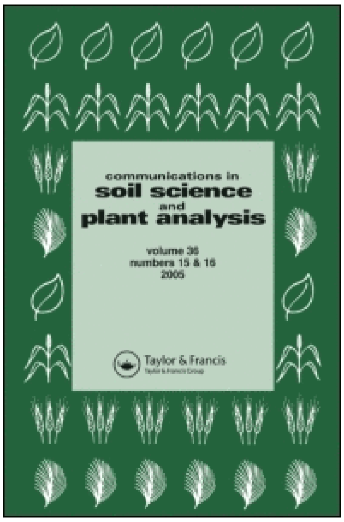


## Communications in Soil Science and Plant Analysis

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597241

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Online publication date: 06 November 2010

**To cite this Article** Zhao, Kun , Li, Shi-Qing , Xu, Bing-Cheng , Lu, Hong-Ling and Li, Sheng-Xiu(2010) 'Comparative Study on Disturbed and Undisturbed Soil Sample Incubation for Estimating Soil Nitrogen-Supplying Capacity', Communications in Soil Science and Plant Analysis, 41: 20, 2371 — 2382

**To link to this Article: DOI:** 10.1080/00103624.2010.511370

**URL:** http://dx.doi.org/10.1080/00103624.2010.511370

*Communications in Soil Science and Plant Analysis*, 41:2371–2382, 2010

ISSN: 0010-3624 print / 1532-2416 online
DOI: 10.1080/00103624.2010.511370



# Comparative Study on Disturbed and Undisturbed Soil Sample Incubation for Estimating Soil Nitrogen-Supplying Capacity

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*Application of nitrogen (N) fertilizers without knowing the N-supplying capacity of soils may lead to low N use efficiency, uneconomical crop production, and pollution of the environment. Based on the results from pot experiments treated with soil initial nitrate leaching and native soil, long-term alternate leaching aerobic incubation was conducted to study the disturbed and undisturbed soil N-supplying capacity of surface soil samples in 11 sites with different fertilities on the Loess Plateau. The results indicated that the entire indexes and ryegrass (Lolium perenne) uptake N with soil initial nitrate leaching showed a better correlation than that without soil initial nitrate leaching. Except the correlation coefficients for soil initial nitrate ($NO_3^-$)-N and mineral N extracted by calcium chloride ($CaCl_2$) before aerobic incubation with ryegrass uptake without soil initial nitrate leaching, the correlation coefficients for soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation with ryegrass uptake with soil initial nitrate leaching and those for mineralizable N extracted by aerobic incubation, soil initial mineral N and mineralizable N extracted by aerobic incubation, potentially mineralizable N ($N_0$) and soil initial mineral N + $N_0$ with ryegrass uptake N under the two cases in disturbed treatment were all higher than those in undisturbed treatment. We concluded that $NO_3^-$-N in soil extracted by $CaCl_2$ before aerobic incubation can reflect soil N-supplying capacity but cannot reflect soil potential N-supplying capacity. Without soil initial nitrate leaching, the effect of disturbed and undisturbed soil samples incubated under laboratory conditions for estimating soil N-supplying capacity was not good; however, with soil initial nitrate leaching, this method could give better results for soil N-supplying capacity. Based on the results from pot experiments treated with soil initial nitrate leaching and native soil, the mineralization of disturbed soil samples can give provide better results for predicting soil N-supplying*

Received 23 February 2009; accepted 12 January 2010.
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*capacity for in situ structure soil conditions on the Loess Plateau than undisturbed soil samples.*

**Keywords** Crop N uptake, disturbed soil, long-term alternate leaching aerobic incubation, soil mineralization N, soil N-supplying capacity, undisturbed soil



## Introduction

Nitrogen (N) is important for crop production in the semi-arid Loess Plateau, and available N is the source that crops can use directly. It is important to predict the quantity of soil N available to plants with reasonable accuracy and thus optimize the quantity of N fertilizer applied. Soil N-supplying capacity is an important parameter for evaluating soil N-supplying levels and determining the amount of N fertilizer application.

There exists a wide variety of chemical and biological methods to assess N-supplying capacity in the laboratory and in situ (Binkley and Hart 1989). Biological methods are more reasonable because the release of mineral N during incubation processes is similar to its release during crop growth seasons in the field due to the similar microbial environments. Biological methods are normally taken as standard methods for evaluating soil N-supplying capacities.

Stanford and Smith (1972) developed a long-term aerobic laboratory incubation method for both ammonium ($NH_4^+$)-N and nitrate ($NO_3^-$)-N using ideal temperature and moisture conditions for N mineralization and nitrification. Their method called for N elution over successive weeks following laboratory incubation at 35 °C for a total of 30 weeks. The fraction of $N_0$ was found to comprise between 5 and 40% of the total N contained in the soil, and the most reliable estimate of the rate constant $k$ was $0.54 \pm 0.009$ per week, meaning that N was released at an average rate of 54% of the total soil N per week (Stanford and Smith 1972). Many studies indicated that $N_0$ and $k$ could reflect soil potential N-supplying capacity (Wang 1986; Tao, Wu, and Fang 1993). Although this incubation procedure and concept has been used often (e.g., Juma, Paul, and Mary 1984; Nordmeyer and Richter 1985; Hadas et al. 1986; Cabrera 1993; Ye, Zhang, and Li 2001; Li, Wang, and Li 2003), criticism has arisen concerning mathematical as well as experimental aspects of parameter estimation (see Skjemstad, Vallis, and Myers 1988). However, Li et al. (1992) pointed out that long-term alternate leaching aerobic incubation had the following characteristics: no need to add water during the whole incubation period, and toxic substances and $NO_3^-$-N formed could be removed by leaching. Moreover, it could be used to reflect the recent soil N-supplying level and potential.

Soil N-supplying capacity studies provide important information in the area of the Loess Plateau. The results accord with the reality can offer scientists and farmers a new and valid way for reasonable evaluation and decision making. Most of the previous analyses of N-supplying capacity using long-term alternate leaching aerobic incubation methods in this area have focused on disturbed soil samples (D; e.g., Fu and Li 1992; Jin et al. 2007) but few considered using undisturbed soil samples (UD). It was important to test the soil N-supplying capacity for disturbed and undisturbed soils. The selected study areas were typical of Loess Plateau fields regarding soil type. Hence it is possible to generalize the results of this study for a majority of the Loess Plateau in northwest China.

The purposes of the present study were to improve the capacity to predict variation in N mineralization and to develop better methods of assessing soil N-supplying capacity to guide management. Accordingly, based on the results from pot experiments treated with soil initial nitrate leaching and native soil, we experimentally tested whether the mineralization on D or UD soil samples can adequately predict soil N-supplying capacity for in situ structure soil conditions on the Loess Plateau and to provide a basis for the pool of soil N-supplying capacity under UD conditions.

## Materials and Methods

### *Study Site Description*

The soils used in the study are from the Loess Plateau covering the total of semi-arid area in the north to the subhumid region in the south (Figure 1). The average annual temperature is 3.6–14.3 °C, and the yearly mean precipitation is 150–750 mm. Eleven soil samples in the tillage layer (0–20 cm) from north (Shenmu) to south (Yangling), in the province of Shaanxi, were used based on soil types. Soil types were Ust Sandic Entisols, Los Orthic Entisols, Hap Ustic Isohumisols, and Eum Orthic Anthrosols at Sites 1 to 11 (Table 1; Gong 1999), respectively. The selected plots in these 11 soils were in bare fallow at the time of sampling (at the end of April 2007).

### *Soil Sampling*

Ten to 12 subsamples of moist soil from the 0–20 cm layer were taken from each site using an auger method and combined to make a bulk sample of approximately 40 kg. Each soil sample was passed through a 6-mm sieve to remove stones, coarse root fragments, and stubble and then mixed thoroughly. After that, a part of (about 5 kg) the moist soil (fresh soil) was passed through a 2-mm sieve and air-dried to measure soil N-supplying capacity. The rest of the soil which was passed through the 6-mm sieve and air-dried for D soil pot experiments.

We collected both D and UD soil samples in all sites, and there were three replicates for ech sample.

Soil cores were obtained using polymethyl methacrylate (PMMA) tubes and polyvinyl chloride (PVC) cylinders for UD studies. For each site, three PMMA tubes with 5 cm inside diameter and 20 cm length were used for incubation experiments, and three PVC cylinders with 10 cm inside diameter were used for pot experiment. The PMMA tubes and PVC

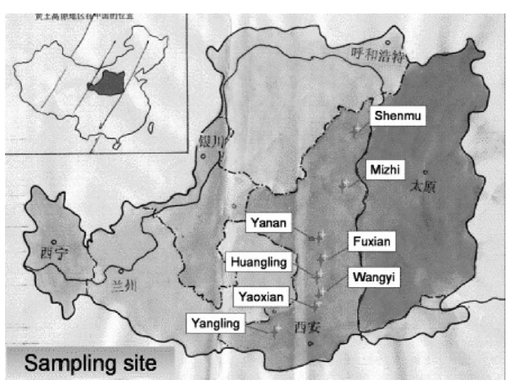


**Figure 1.** Soil sampling scheme.

**Table 1**
Basic properties of soil samples at each site

| No. | Location | Soil type | Organic matter ($g.kg^{-1}$) | Total N ($g.kg^{-1}$) | Mineral N ($\mu g.g^{-1}$) | Available P ($\mu g.g^{-1}$) | pH ($H_2O$) |
|---|---|---|---|---|---|---|---|
| 1 | Shenmu | USE | 9.53 | 0.68 | 19.15 | 15.40 | 8.1 |
| 2 | Shenmu | USE | 8.83 | 0.36 | 15.31 | 92.43 | 8.0 |
| 3 | Mizhi | USE | 3.43 | 0.19 | 13.25 | 89.83 | 8.3 |
| 4 | Mizhi | USE | 12.40 | 0.24 | 8.23 | 65.13 | 8.2 |
| 5 | Yanan | LOE | 3.77 | 0.67 | 24.22 | 53.90 | 8.4 |
| 6 | Fuxian | LOE | 14.80 | 0.54 | 14.00 | 130.43 | 8.3 |
| 7 | Huangling | HUI | 11.47 | 0.72 | 11.45 | 86.90 | 8.4 |
| 8 | Wangyi | HUI | 10.60 | 0.59 | 5.47 | 114.50 | 8.3 |
| 9 | Yaoxian | HUI | 11.03 | 0.65 | 10.91 | 101.57 | 8.1 |
| 10 | Yangling | EOA | 17.67 | 0.98 | 12.68 | 157.47 | 7.9 |
| 11 | Yangling | EOA | 14.13 | 0.77 | 30.19 | 103.37 | 8.0 |
| Mean | | | 10.70 | 0.58 | 14.99 | 91.90 | 8.2 |

*Note.* USE = Ust Sandic Entisols; LOE = Los Orthic Entisols; HUI = Hap Ustic Isohumisols; EOA = Eum Orthic Anthrosols.



cylinders were pushed into the soil vertically using a hammer and wood block. Each core was carefully lifted from the ground using a shovel underneath the core to prevent spillage. Efforts were made to have the same volume of soil in each cylinder. The cylinders were capped on both ends, marked at the top, stored upright, and brought to the lab. Soil samples for incubation were stored at 0–4 °C prior to the incubation.

At each site, soil was collected from the top 20 cm, air-dried, sieved (2 mm), and prepared for initial physical and chemical analyses. Some measurements were done using field-moist soil (Table 1).

### *Sample Incubation*

*D Soil Sample Incubation.* Incubations were carried out over 30 weeks (long-term alternate leaching aerobic incubation) according to Stanford and Smith (1972). Controlled moisture and temperature conditions in laboratory incubations isolate substrate quality from other controls over soil N mineralization. Soil samples from each site were gently sieved through a 2-mm sieve and mixed with acid-washed quartz sieved through 2 mm $< d <$ 3 mm sieve in a 1:1 ratio (15 g soil + 15 g quartz; Stanford and Smith 1972). The addition of quartz was used to maintain adequate drainage after repacking the soil columns.

The soil samples, contained in 33 cylinders, were initially leached and then incubated; 100 mL 0.01 mol $L^{-1}$ calcium chloride ($CaCl_2$) was used to leach the initial mineralized N in soil, and then 25 mL N-free nutrient solution (0.002 M calcium sulfate [$CaSO_4 \cdot 2H_2O$], 0.002 M magnesium sulfate [$MgSO_4 \cdot 7H_2O$], 0.005 M calcium phosphate [$Ca(H_2PO_4)_2 \cdot H_2O$], 0.0025 M potassium sulfate [$K_2SO_4$]) were added to avoid nutrient deficiency. A moisture tension of 80 KPa at the bottom of the cores was achieved using a water circulation multifunction vacuum pump, which was connected to the bottom of the soil cores. Samples were covered with plastic wrap to prevent moisture loss and provide better aeration. The incubation chamber temperature was maintained at 35 °C (±1 °C). Samples were leached during weeks 2, 4, 8, 12, 16, 22, and 30.

The concentration of $NO_3^-$-N and $NH_4^+$-N was determined using a flow injection procedure (AutoAnalyzer 3 Digital Colorimeter, Bran Luebbe, Norderstedt). All inorganic N concentrations are expressed on an oven-dry, elemental N basis. Solution concentrations were expressed as mg N·$g^{-1}$ soil N to normalize differences in total soil N among samples. All values refer to net N mineralization. Cumulative mineralization for a given period was the sum of N mineralized for that period and the previous periods.

*UD Soil Sample Incubation.* In the laboratory, 33 PVC cylinders were constructed for UD treatments by removing the surface 4 cm of soil from a given cylinder, and an appropriate amount of acid-washed quartz was added to prevent soil from scattering while leaching. A folium of fiberglass and an appropriate amount of acid-washed quartz were then placed at the bottom of each cylinder and pressed slightly to ensure soil-to-resin contact and secured with a perforated rubber plug. The top plastic cap was secured by a rubber band to minimize excessive drying and was removed later. The quantities of $CaCl_2$ and N-free nutrient solution were both 17-fold of D soil samples, based on dry weight. Other leaching and measurement methods were the same as those described in D soil sample incubation.

### Pot Experiment

The pot experiments were performed on 11 soil sites with and without soil initial nitrate leachings. The pots of each soil were sown with ryegrass.

*D Soil Pot Experiment.* The pots used were PVC cylinders (10 cm in diameter and 20.0 cm in depth). Six replicate pots of each soil were filled to within 1.5–2.0 cm of the top and the pots were tapped several times to consolidate the soil. The weight of air-dried soil per pot was 2.0 kg. After filling soil in pots, three pots of each soil sample were leached at random, and the initial nitrates in the soil were removed with distilled water until the leached liquid did not produce a red color when reacted with the nitro indicator (Dickson and Aitken 1993), and three pots of each soil were used without leaching. The purpose of leaching the soils to remove mineral N in calcareous soils, mainly the nitrate, was to assure that the crop uptake N fully reflected the amount of mineralized N in the soils (Ju and Li 1996), because the nitrate in soils interfered with the crop uptake of N. After leaching, irrigating was done on the 66 pots without leaching the soil nitrate with 400 mL of distilled water in order to keep the water content similar to that of pots with leaching.

Every pot of each soil sample was sown with ryegrass at a rate of 30 seeds per pot at a depth of 1.5 cm. After emergence, the number of seedlings was reduced to 20 per pot. Free-N nutrient solution (the same as for incubation) was irrigated at the seedling stage, in order to ensure that the other nutrients, except for N, were not a constraint on ryegrass growth. The free-N solution provided 0.15 g $P_2O_5$ $kg^{-1}$ soil per pot. Pots were irrigated with distilled water during plant growth, depending on the rate of water loss. The liquids leaked into the base pots were returned to the pots before irrigating and then they were irrigated with an appropriate amount of water.

The ryegrass with leaching and without soil initial nitrate leaching was grown on May 17, 2007, and harvested on five occasions by cutting 1.5 cm above the soil surface. The last harvest was carried out on June 10, 2008, by cutting as close to the surface of the soil as possible. The roots were carefully washed out of the soil for analysis. All the plant materials were dried and weighed, and the total N content of the above-ground part was determined.

*UD Soil Pot Experiment.* The UD soil pot experiments were managed and harvested in the same manner as that of D soil samples.

### *Chemical and Physical Analysis*

Soil water content was gravimetrically determined on the remaining soil sample following drying at 105 °C for 24 h. Soil pH was potentiometrically measured at a soil:water ratio of 1:5, and extractable phosphorus by the sodium bicarbonate ($NaHCO_3$) method (Olsen P method). Organic carbon (C) was determined by the potassium dichromate–outer heating method. The Kjeldahl method was developed to determine total N (KJELTEC 2300 type fully automatic azotometer; Foss Tecator AB, Hoganas Sweden). Fresh soil samples (10.0 g) were extracted with 50 mL 1 M potassium chloride (KCl) for 1 h with continuous shaking at 220 rev·min$^{-1}$. $NO_3^-$-N and $NH_4^+$-N were determined using a flow injection procedure (AutoAnalyzer 3 Digital Colorimeter).



### *Statistical Method*

Most of the results shown in the present article are the mean of three replicate analyses. Correlation analysis was applied for the values obtained by the long-term alternate leaching aerobic method and uptake N of ryegrass using Origin statistics software (OriginLab Corporation). Correlation analysis was also made for mineralized N during the process of incubation and crop uptake N with soil initial nitrate leaching and for the mineralized N + soil initial nitrate and crop uptake N without soil initial nitrate leaching.

## Results

Figure 2 presents the cumulative mineralized N of alternative leaching 30 weeks' aerobic incubation averaged among the 11 soil samples. Comparing D and UD treatments for both unleaching (UL) and leaching (L) soil samples, the amount of N mineralized in D soil samples was larger than that in UD soil samples, as found by other authors (Cabrera and Kissel 1988; Sierra 1992; Stenger, Priesack, and Beese 1995; Ringuelet and Bachmeier 2002). This result indicated that any disturbance introduced by soil preparation has a strong influence on subsequent N mineralization. Moreover, the soil initial mineral N leached from D soil samples was larger than that in UD soil samples, which may result from the compact ability of UD soil samples. The UD soil samples were compact and difficult to leach, and the D soil samples were introduced by drying and rewetting and easier to leach.

The cumulative ryegrass N uptake averaged among the 11 soils for the pot experiments is summarized in Figure 3. Measurement of ryegrass N uptake during the cutting period revealed differences among different treatments. The ryegrass N uptake was at its highest in the D-UL treatment and was not significantly different than the other three pot treatments. For all the four treatments, the fourth cutting period experienced the lowest N uptake. The main reason was that the temperature was low and so was the biomass.

Correlation analysis of indexes with crop N uptake is an effective way to evaluate the indexes of mineralizable N obtained from lab experiments. So the correlation coefficients of the indexes obtained using a long-term alternate leaching aerobic incubation method

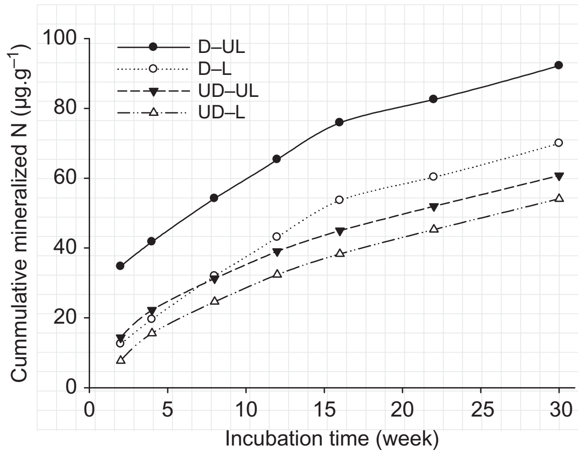


**Figure 2.** Cumulative N mineralization averaged among the 11 soils versus incubation time for different soil treatments. D-UL = disturbed soil sample with initial mineral N; D-L = disturbed soil sample without initial mineral N; UD-UL = undisturbed soil sample with initial mineral N; UD-L = undisturbed soil sample without initial mineral N.

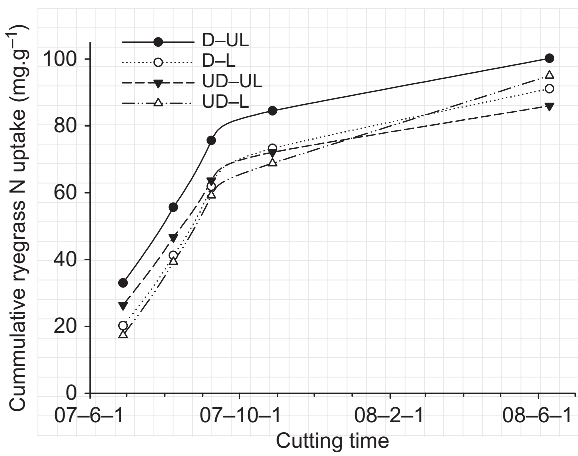


**Figure 3.** Cumulative ryegrass N uptake averaged among the 11 soils versus cutting time for different pot treatments. D-UL = disturbed soil sample without leaching initial nitrate; D-L = disturbed soil sample with leaching initial nitrate; D-UL = undisturbed soil sample without leaching initial nitrate; UD-L = undisturbed soil sample with leaching initial nitrate.

with N uptake by pot crops were chosen to be analyzed to ensure the reliability of these indexes for estimating the size of the soil mineralizable N pool. Analytical results are listed in Tables 2, 3, and 4.

## *Soil Initial Mineral N and Soil N-Supplying Capacity*

For D soil samples, when soil initial $NO_3^-$-N was not leached, soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation were positively related with ryegrass N uptake, and the correlation coefficients were 0.604 and 0.597, respectively. When

**Table 2**
Correlation coefficient for soil initial mineral N with ryegrass uptake N

| | D | | UD | |
|---|---|---|---|---|
| Index | UL | L | UL | L |
| Soil initial nitrate | 0.604 | 0.689 | 0.856 | 0.613 |
| Soil initial mineral N | 0.597 | 0.671 | 0.862 | 0.607 |

*Note.* Significance level: $r_{0.05}(9) = 0.602$, $r_{0.01}(9) = 0.735$.

**Table 3**
Correlation coefficient for cumulative mineralized N with ryegrass uptake N

| | D | | UD | |
|---|---|---|---|---|
| Index | UL | L | UL | L |
| $N_m$ | 0.789 | 0.860 | 0.410 | 0.718 |
| Soil initial mineral N + $N_m$ | 0.818 | 0.897 | 0.553 | 0.782 |

*Note.* $N_m$ = mineralized N after 30 weeks' incubation.
Significance level: $r_{0.05}(9) = 0.602$, $r_{0.01}(9) = 0.735$.

**Table 4**
Correlation coefficient for potentially mineralizable N with ryegrass uptake N

| | D | | UD | |
|---|---|---|---|---|
| Index | UL | L | UL | L |
| $N_0$ | 0.827 | 0.855 | 0.492 | 0.640 |
| Soil initial mineral N + $N_0$ | 0.831 | 0.897 | 0.419 | 0.688 |

*Note.* $N_0$ = Potentially mineralizable N.
Significance level: $r_{0.05}(9) = 0.602$, $r_{0.01}(9) = 0.735$.



initial $NO_3^-$-N was leached, the correlation coefficients for soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation with ryegrass N uptake were increased to 0.689 and 0.671, respectively, and both reached a 5% significance level. For UD soil samples, when initial $NO_3^-$-N was not leached, soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation were closely related with ryegrass N uptake, the correlation coefficients were 0.856 and 0.862, respectively, and both reached a 1% significance level. However, when initial $NO_3^-$-N was leached, the correlation coefficients for soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation with ryegrass N uptake were decreased to 0.613 and 0.607, respectively, and only reached a 5% significance level (Table 2).

### *Cumulative Mineralized N and Soil N-Supplying Capacity*

For D soil samples, the mineralized N obtained by aerobic incubation had a remarkably positive correlation with crop uptake N without leaching the soil initial nitrate (r = 0.789,

$P < 0.01$). The correlation coefficient was higher for mineralized N and crop uptake N with initial nitrate leaching ($r = 0.860$, $P < 0.01$). For UD soil samples, the relationship between mineralizable N extracted by aerobic incubation with ryegrass uptake N without leaching the soil initial nitrate was not good ($r = 0.410$, $P > 0.05$). The correlation coefficient for mineralizable N extracted by aerobic incubation with ryegrass uptake N with leaching the soil initial nitrate was 0.718 and reached a 5% significance level, lower than that for D soil samples. For both D and UD soil samples, correlation coefficients of the sum of soil initial mineral N and mineralizable N extracted by aerobic incubation with ryegrass uptake N were higher than that of the latter two (Table 3).

### *Potentially Mineralizable N ($N_0$) and Soil N-Supplying Capacity*

The correlation analysis (Table 4) showed that for D soil samples, $N_0$ was significantly correlated with ryegrass N uptake without soil initial nitrate leaching ($r = 0.827$, $P < 0.01$). The correlation coefficient reached up to 0.855 for $N_0$ and ryegrass N uptake with soil initial nitrate leaching. And both for soil initial nitrate leaching and unleaching pot experiments, the correlation coefficient of $N_0$ and soil initial mineral N + $N_0$ with ryegrass N uptake was higher for D than UD. Moreover, the relationship of $N_0$ and soil initial mineral N + $N_0$ with ryegrass N uptake without soil initial nitrate leaching was not good for UD soil samples. Thus, for D soil samples, $N_0$ can be used to evaluate mineralized N of soils and $N_0$ + initial mineral N (mainly nitrate in dryland calcareous soils) can be used to evaluate the soil N-supplying capacity. However, for UD soil samples $N_0$ + initial mineral N cannot be used to evaluate the soil N-supplying capacity.

## Discussion

Long-term alternate leaching aerobic incubation is good for continuous mineralization processes due to leaching and removing the toxic substances and nitrate, it does not require the addition of water during incubation, and it can reflect the recent N-supplying capacity (S. X. Li et al. 1992). The main defect of this method is that the soils need a long time for incubation, which is complicated. But it is a standard method as an index for reflecting the soil N-supplying capacity. It is also taken as a reference method for other methods. The value of $N_0$ was obtained by this method as the soil N-supplying capacity volume index.

The plant absorption method has been generally accepted to measure plant N uptake in the whole growing season (Yan and Wang 2005). It is assumed that plant N uptake was the sum of N mineralized and mineral *N* change during the plant growing season when there is no external input of N (Zhou 1988). Mineralized N calculated by this method could comprehensively reflect the effect of a special soil–plant–environment system on the soil organic N mineralization process. Therefore, it is often chosen as a standard method compared to other methods.

Soil mineral N can reflect soil N-supplying capacity. Ammonium N in calcareous soil could easily convert to nitrate N (Hu, Li, and Hao 2000), so ammonium N content is at a low and stable level, around 4 $\mu g.g^{-1}$. Nitrate N content was not only high in different soil types but there are big differences among them. Therefore, soil initial nitrate as an indicator for estimating soil N-supplying capacity has captured the attention of researchers (Ye and Li 2002) and was widely used as index for evaluating soil current N-supplying capacity (Li et al. 1992; Hu, Li, and Hao 2000). Our results showed that if the soil initial $NO_3^-$-N is high, $NO_3^-$-N in soil extracted by $CaCl_2$ before aerobic incubation can reflect soil N-supplying capacity (Hu, Li, and Hao 2000; Ye and Li 2002) but cannot reflect soil potential N-supplying capacity.

Inorganic N absorbed and used by crops was not only from the soil initial mineral N pool but also from mineralized N through soil organic N mineralization (Ju, Liu, and Zhang 2004). Therefore, it is essential to consider the effect of both soil initial mineral N and mineralizable N on crop N uptake in objectively and generally estimating soil N-supplying status and determining a satisfactory index for soil N-supplying capacity. The simplest and most fundamental way to prove whether the mineralized N obtained by the long-term alternate leaching aerobic incubation method was meaningful and could reflect N-supplying capacity of soils was to exclude the interference of $NO_3^-$-N to create a condition in which mineralizable N could play a role. In this way we could test the real value of the method. Based on this consideration, the pot experiment was divided into two treatments because the initial nitrate was the main part of the calcareous soil initial mineral N: (1) leaching the soil initial nitrate; (2) not leaching the soil initial nitrate. The crop uptake N of the former reflects the soil mineralized N without including the soil initial nitrate. The latter reflects the soil N-supplying capacity. Therefore, the pot experiments were used to evaluate D and UD soil sample incubation methods with crop uptake N under the two cases as references. An ideal method can reflect both soil mineralized N and soil N-supplying capacity but most are used to measure soil mineralized N.



We could assume that if the correlation coefficient for the mineralized N and crop N uptake with soil initial nitrate leaching improved obviously, this would prove that this method could reflect the real process of soil organic mineralization. In other words, the poor effects of mineralizable N without soil initial nitrate leaching reflecting soil N capacity were influenced and disturbed by initial nitrate. On the contrary, if the mineralized N had no correlation with crop N uptake without soil initial nitrate leaching, it proves that this method cannot reflect the real process of soil organic mineralization. The present study showed that both for D and UD soil samples, when initial $NO_3^-$-N were leached, the correlation coefficients for mineralizable N extracted by aerobic incubation, soil initial mineral N and mineralizable N extracted by aerobic incubation with ryegrass N uptake were significantly increased. The results indicated that without soil initial nitrate leaching, this method cannot reflect soil N-supplying capacity but it can fully reflect it with soil initial nitrate leaching.

Nitrogen mineralization potential is referred to the maximum amount of N extracted from soil N in some conditions, and could reflect N supply capacity and intensity (Lv et al. 1996). $N_0$ and ryegrass N uptake under the two situations were both strongly correlated. Soil initial mineral N + $N_0$ had poorer correlations with ryegrass N uptake without soil initial nitrate leaching than the other situation. Therefore, the whole results showed that taken ryegrass N uptake with soil initial nitrate leaching as reference, $N_0$ can be used to evaluate mineralized N of soils. When soil initial nitrate was not leached, soil initial mineral N + $N_0$ could not be used to evaluate the soil N-supplying capacity; however, with soil initial nitrate leaching, soil initial mineral N + $N_0$ could provide better results for soil N-supplying capacity.

Comparing D and UD treatments, the correlation coefficients for soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation with ryegrass uptake without soil initial nitrate leaching in D treatment (r = 0.604 and 0.597) were lower than those in UD treatment (r = 0.856 and 0.862). However, the correlation coefficients for soil initial $NO_3^-$-N and mineral N extracted by $CaCl_2$ before aerobic incubation with ryegrass uptake with soil initial nitrate leaching and the correlation coefficients for mineralizable N extracted by aerobic incubation, soil initial mineral N and mineralizable N extracted by aerobic incubation, $N_0$ and soil initial mineral N + $N_0$ with ryegrass uptake N under the two cases in D treatment were all higher than those in UD treatment. The results showed that based on the results from pot experiments the mineralization of D soil samples can provide better results for predicting soil N-supplying capacity on the Loess Plateau than UD soil samples.

A review of the methods for evaluating calcareous soil N-supplying capacity included a variety of factors. The key part was that it is convenient and simple and can reflect the actual situation. There are many constraints for using UD soil samples, such as the limited number of samples that can be studied and the difficulties associated with the extraction of mineral N during the incubation period.

## Conclusions

On the basis of our results it can be inferred that $NO_3^-$-N in soil extracted by $CaCl_2$ before aerobic incubation has a good soil N index, reflecting soil N-supplying capacity; however, it is difficult to reflect soil potential N-supplying capacity. Without soil initial nitrate leaching this incubation method cannot reflect soil N-supplying capacity, but the method can reflect it with soil initial nitrate leaching. Based on the results from pot experiments treated with soil initial nitrate leaching and native soil, the mineralization on D soil samples can provide better results for predicting soil N-supplying capacity for in situ structure soil conditions on the Loess Plateau than UD soil samples.



## Acknowledgments

This work was supported by the National Basic Research Program of China (2009CB118604), the National Natural Science Foundation of China (90502006), and the Natural Science Foundation of State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (10502-Z04).

## References

Binkley, D., and S. C. Hart. 1989. The components of nitrogen availability assessments in forest soils. In *Advances in soil science*, Vol. 10, ed. B. Stewart, 57–103. New York: Springer Verlag.

Cabrera, M. L. 1993. Modeling the flush of nitrogen mineralization caused by drying and rewetting soils. *Soil Science Society of America Journal* 57:63–66.

Cabrera, M. L., and D. E. Kissel. 1988. Potentially mineralizable nitrogen in disturbed and undisturbed soil samples. *Soil Science Society of America Journal* 52:1010–1015.

Dickson, T., and R. L. Aitken. 1993. Prediction of nitrogen fertilizer requirements of maize in subtropic Queensland. *Australian Journal of Experimental Agriculture* 33:53–58.

Fu, H. F., and S. X. Li. 1992. Soil nitrogen mineralization and soil N-supplying capacities. The relationship between mineralized N and plant uptake N. *Journal of Northwest Sci-Tech University of Agriculture and Forest (Natural Science Edition)* 20(Suppl.):53–58.

Gong, Z. T. 1999. *Chinese soil taxonomy*. Beijing: Science Press.

Hadas, A., S. Feigenbaum, A. Feigin, and R. Portnoy. 1986. Nitrogen mineralization in profiles of differently managed soil types. *Soil Science Society of America Journal* 50:314–319.

Hu, T. T., S. X. Li, and Q. K. Hao. 2000. The relationship between mineral N and mineralization N with soil N supplying capacity in arid area. *Journal of Soil Water Conservation* 14 (4):83–86.

Jin, F. H., S. Q. Li, H. L. Lu, and S. X. Li. 2007. Estimation of biological methods on assessing soil nitrogen-supplying capacity in calcareous soil. *Scientia Agricultura Sinica* 40:1422–1431.

Ju, X. T., and S. X. Li. 1996. The contribution of mineralizable soil N to crop N uptake. *Agricultural Research in the Arid Areas* 14 (4):29–33.

Ju, X. T., X. J. Liu, and F. S. Zhang. 2004. Effects of long-term fertilization on soil organic nitrogen fractions. *Scientia Agricultura Sinica* 37:87–91.

Juma, N. G., E. A. Paul, and B. Mary. 1984. Kinetic analysis of net nitrogen mineralization in soil. *Soil Science Society of America Journal* 48:753–757.

Li, J. M., Z. H. Wang, and S. X. Li. 2003. Significance of soil organic matter, total N and mineralizable nitrogen in reflecting soil N supplying capacity. *Acta Pedologica Sinica* 40 (2):232–237.

Li, S. X., H. F. Fu, J. Z. Xiao, and H. L. Yuan. 1992. The effectiveness of several methods determining soil available or potentially available N in reflecting dryland soil N supply-capacities. *Agricultural Research in the Arid Areas* 10:72–81.

Lv, S. L., X. R. Yang, Y. D. Zhang, and M. Wu. 1996. Nitrogen mineralization potential and the forecast of the content of nitrogen supplied in the soils of Shanxi province. *Scientia Agricultura Sinica* 29 (1):21–26.

Nordmeyer, H., and J. Richter. 1985. Incubation experiments on nitrogen mineralization in loess and sandy soils. *Plant Soil* 83:433–445.

Ringuelet, A., and O. A. Bachmeier. 2002. Kinetics of soil nitrogen mineralization from undisturbed and disturbed soil. *Communications in Soil Science and Plant Analysis* 33:3703–3721.

Sierra, J. 1992. Relationship between mineral N contents and N mineralization rate in disturbed and undisturbed soil samples incubated under field and laboratory conditions. *Australian Journal of Soil Research* 30:477–492.

Skjemstad, J. O., I. Vallis, and R. J. K. Myers. 1988. Decomposition of soil organic nitrogen. In *Advance in nitrogen cycling in agricultural ecosystems*, ed. J. Wilson, 134–144. Wallingford, UK: CAB International.

Stanford, G., and S. J. Smith. 1972. Nitrogen mineralization potentials of soils. *Soil Science Society of America Proceedings* 36:465–472.

Stenger, R., E. Priesack, and F. Beese. 1995. Rates of net nitrogen mineralization in disturbed and undisturbed soils. *Plant and Soil* 171:323–332.

Tao, Q. N., L. H. Wu, and P. Fang. 1993. Study on mineralization rate of nitrogen in paddy soils. *Acta Pedologica Sinica* 8:237–244.

Wang, Y. Q. 1986. Nitrogen mineralization potential pf loess soil, cinnamon soil and fluvo-aquic soil in China. *Acta Pedologica Sinica* 2:19.

Yan, D. Z., and J. D. Wang. 2005. Methods for studying soil nitrogen supply capacity. *Soils* 37:20–24.

Ye, Y. L., and S. X. Li. 2002. Influence of initial nitrate-nitrogen on methods of measuring soil nitrogen supplying capacity in calcareous soils. *Plant Nutrition and Fertilizer Science* 8 (3):310–317.

Ye, Y. L., F. S. Zhang, and S. X. Li. 2001. Study on soil nitrogen supplying indexes. *Chinese Journal of Soil Science* 32 (6):273–277.

Zhou, M. Z. 1988. *Measures of soil fertility and fertilization according to soil test*. Beijing: China Agriculture Press.