Research note

Effect of Sample Treatments on Estimation of *in situ* Soil Available Nitrogen of the Fushan Hardwood Forest

Fu-Wen Horng,[1,3] Ren-Chin Huang[2]

[Summary]

Three treatments were tested on a warm (July) and a cold (March) sampling dates for the determination of *in situ* inorganic nitrogen of surface soil in the Fushan hardwood forest. The commonly used KCl solution was applied as the extraction solution on 3 separate occasions before the determination. According to the results, immediate extraction of soil samples gives the best estimation of NO_3^--N, NH_4^+-N, and total inorganic N concentrations in soil. Keeping soil samples in the extraction solution and quickly sending them back to the laboratory can produce values close to those of field extraction. This is better than those staying overnight, then being transported, and extracted in the laboratory. Deviations from the estimation by *in situ* extraction are more serious in the warm than in the cold season. Therefore, immediate field extraction is recommended for determination of *in situ* inorganic nitrogen.

Key words: soil available nitrogen, soil extract, soil treatment.

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研究簡報

土壤樣本處理對於福山闊葉林土壤現地有效氮估算的影響

洪富文[1,3] 黃仁欽[2]

摘要

在一個暖月(7月)與冷月(3月)採取福山闊葉林的表層土壤，測定現存土壤有效態氮。三種方法分別爲1 N KCl溶液在現場抽出(處理1)，或在現場浸泡後回試驗室抽出(處理2)，或留存於室溫回試驗室後抽出(處理3)。抽出液則測定所含NO_3^--N，NH_4^+-N，以及全無機氮的含量。結果顯示處理2比處理3的值接近處理1的值；暖月的值比冷月的值偏離處理1更大。因此，現場抽出是估算現地土壤有效態氮最好的方法。

[1] 行政院農業委員會林業試驗所育林系，台北市100南海路53號 Division of Silviculture, Taiwan Forestry Research Institute. 53 Nanhai Rd., Taipei 100, Taiwan, ROC.

[2] 台灣大學農業化學系，台北市106羅斯福路四段1號 Department of Agricultural Chemistry, National Taiwan Univ. 1 Sec. 4, Roosevelt Rd., Taipei 106, Taiwan, ROC.

[3] 通訊作者 Corresponding author

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INTRODUCTION

Nitrogen is the most demanded nutrient for plant growth. Most nitrogen in an ecosystem is in the soil and is bound up with organic matter. Before it becomes available for plant utilization, organic-bound nitrogen needs to be transformed into inorganic forms by soil microbes. In general, inorganic nitrogen comprises less than 2-3% of total soil nitrogen, and transformation of organic into inorganic nitrogen by soil microbes is very rapid. If the extraction method and its timing are delayed, the available nitrogen determined may not properly reflect actual *in situ* concentrations and amounts.

Currently the most commonly used method to determine *in situ* available nitrogen in soil is inorganic nitrogen extracted by a 1 N KCl solution (Marrs et al. 1991, Schimel et al. 1992, Barraclough and Puri 1995) or 2 N KCl solution (Binkley and Hart 1989, van Miegroet 1995). However, determination of inorganic nitrogen in the extracted solution requires some sophisticated laboratory instruments, and those are generally not available in the field. During handling and storage before extraction, soil disturbance can stimulate nitrification (Johnson et al. 1991) or ammonification (van Miegroet 1995). Even storing field-moist soils in a refrigerator for short time periods (ranging from 24 h to 4 d) may elevate NO_3^- levels in the extracted solution (Ross and Bartlett 1990). Consequently, soil samples or extracted soil solutions require proper preservation or storage to prevent further changes or modifications before the determination.

This study assessed 3 treatments of soil samples for estimation of inorganic nitrogen in the soil of Fushan forests, considering transportation and equipment difficulty in order to obtain a proper estimation of *in situ* available inorganic nitrogen in this soil.

MATERIALS AND METHODS

The Fushan forest is located in northeastern Taiwan. It is a subtropical Lauro-Fagaceae mixed hardwood forest, and the soil is a lithic dystrochrept with pHs ranging from 3.8 to 5.0, and base saturation below 5% (Lin et al. 1996).

In the Fushan forests, four 20 m × 20 m plots were cut to monitor the effects of patch cutting on forest regeneration and associated soil dynamics in 1992. In July 1995 (summer) and March 1996 (early Spring), a total of 36 soil samples at 0-15 and 15-30 cm depths were collected each time in the plots and nearby intact stands. The soils collected represent a range of topographic positions in the forest, which implies a range of soil nitrogen contents included. Each soil sample was subjected to 3 treatments: (1) Within 4 h after soil sampling, an aliquot of 10 g soil sample was added to 50 mL of 1 N KCl solution and shaken for 1 h, then filtered through Whatman no. 42 filter paper. The extract was stored at 4 °C in a field laboratory. Then it was transported back to the soil laboratory in an ice box the next morning (TR1). (2) A 10 g soil sample was added to a 50 mL 1 N KCl solution, stored at 4 °C in the field laboratory within 4 h after soil sampling, and transported back to the soil laboratory like the TR1 sample, then shaken for 1 h, and filtered (TR2). (3) The untreated

portion of the soil sample was left in the field laboratory overnight. After it was transported back to the soil laboratory the next morning, an aliquot of 10 g was extracted with 50 mL 1 N KCl solution with the same procedure as the other 2 treatments (TR3). It takes 3 h to drive back to the soil laboratory.

All extracts were analyzed with a Fast Ion Analyzer (Lachat) for NH_4^+-N and NO_3^--N concentrations within 48 h. Since there was no specific trend at each separate soil depth, the samples of 0-15 and 15-30 cm depths were pooled together for data analysis.

RESULTS AND DISCUSSION

For the summer samples of July 1995, the NO_3^--N concentrations of the TR2 soils were from 0.92 to 8.41 mg kg^{-1} (Table 1), with most around 0.50-1.50 times those of TR1 (Fig. 1), but the regression line indicates that NO_3^--N concentrations of TR1 and TR2 were close (Fig. 2). However, the NH_4^+-N concentrations of the TR2 soils ranged from 2.97 to 19.03 mg kg^{-1} with most around 0.6-4.0 times those of TR1 soils. When the total inorganic available N was compared, the concentrations of the TR2 soils ranged from 5.76 to 24.55 mg kg^{-1} with most being 0.8-2.5 times those of the TR1 soils (3.12-27.87 mg kg^{-1}) with higher estimations of TR2 soils in lower concentrations and lower estimations of TR2 soils in higher concentrations (Fig. 2). For TR3 soils, NO_3^--N concentrations (2.44-16.63 mg kg^{-1}) were all higher than those of TR1 soils, and reached 4.0-6.0 times their values; NH_4^+-N concentrations ranged from 3.12 to 17.50 mg kg^{-1} and were around 0.3-8.0 times those of TR1 soils. Concentrations of total inorganic available N(8.24-22.53 mg kg^{-1}) of TR3 soils were mostly greater than those of TR1 with some reaching 3.0-5.0 times their values. It is apparent that some significant ammonification of organic N and immobilization (or votilization) of inorganic N went on in the soils of TR2 and TR3, when comparing their NH_4^+-N concentrations with those of TR1 soils (Fig. 2) (Proctor 1989, Ross 1989). At the same time, the relatively small differences of NO_3^--N between soils of TR1 and TR2 as shown by the regression line of TR2 and TR1, indicate that nitrification was not enhanced much by TR2. However, the far greater NO_3^--N concentrations in soils of TR3 than those in TR1 soils (Fig. 2) indicate that great enhancement of nitrification occurred in soil samples merely left overnight. Ross and Bartlett (1990) reported that for spodosols of a high-elevation forest,

Table 1. NO_3^- - N, NH_4^+ - N, and total inorganic N concentrations (mg kg^{-1}) of soil with 3 treatments at 2 sampling dates for Fushan fores soil

Date		NO_3^--N			NH_4^+-N			Total inorganic N		
		TR1[1)]	TR2[2)]	TR3[3)]	TR1	TR2	TR3	TR1	TR2	TR3
July 1995	Mean	3.51	3.33	7.84	5.28	7.58	5.71	8.78	10.91	13.55
	SE	1.81	1.85	3.79	4.08	3.44	2.49	5.00	4.27	3.92
	Range	0.60~7.17	0.92~8.41	2.44~16.63	1.70~23.69	2.97~19.03	3.12~17.50	3.12~27.87	5.76~24.55	8.26~22.53
March 1996	Mean	2.87	3.47	4.21	11.12	13.93	21.13	13.97	17.40	25.34
	SE	1.68	1.93	2.49	4.20	4.01	4.75	4.81	4.94	6.58
	Range	0.90~6.60	1.20~8.10	1.20~10.40	5.10~20.60	6.50~23.20	11.60~34.20	6.00~23.30	7.80~27.40	13.80~41.80

[1)]TR1: treatment 1, *in situ* extraction.

[2)]TR2: treatment 2, extracts were soaked, the extracted in the laboratory.

[3)]TR3: treatment 3, extracts were stored overnight, then extracted in the laboratory.

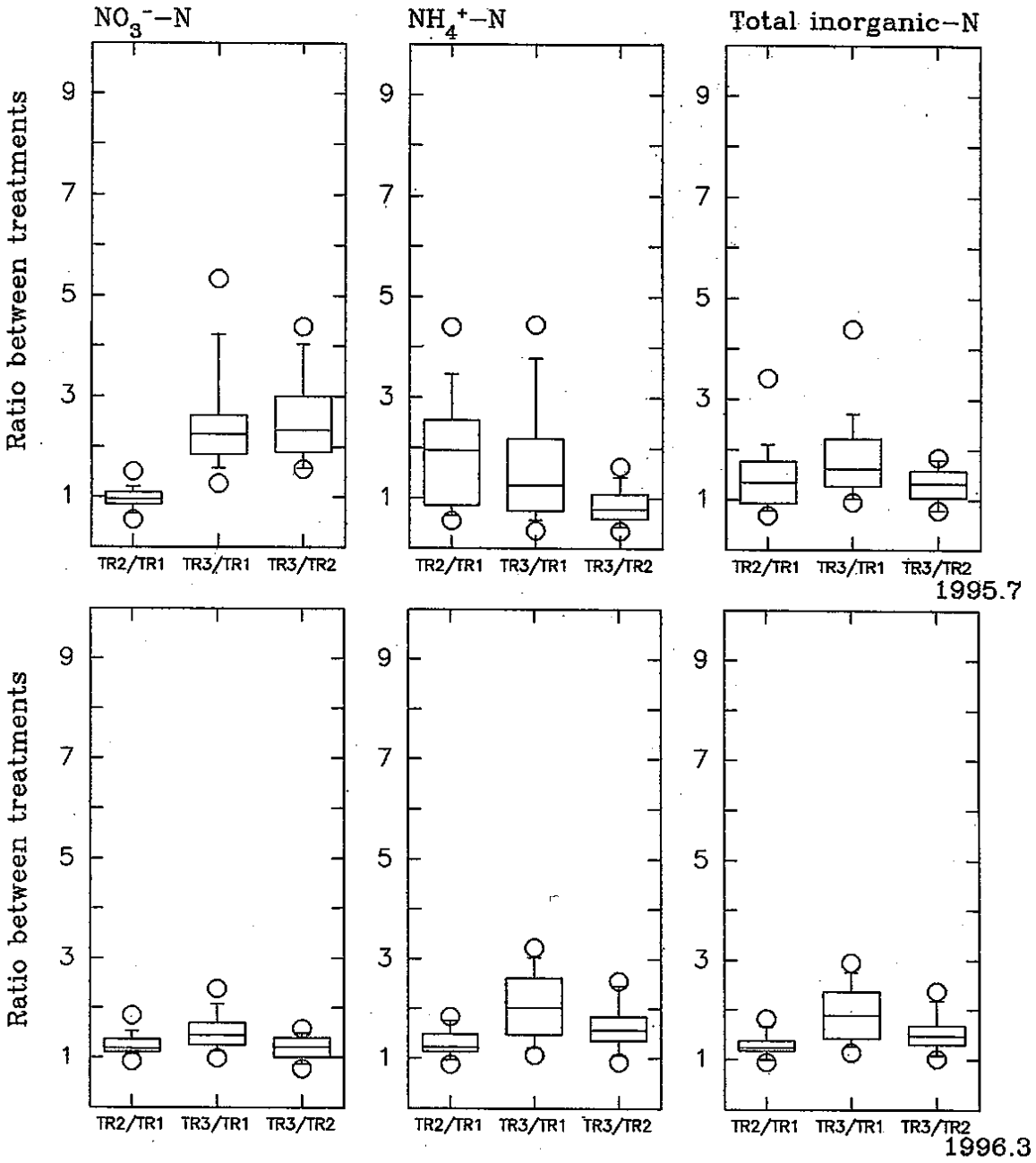

Fig. 1. Ratios between treatments of NO_3^- -N, NH_4^+ -N, and total inorganic N concentrations for soil of a Fushan hardwood forst on 2 sampling dates.

large increases in solution NO_3^- occurred after only 24 h of storage at 3 °C, not to mention the even larger increases after storage at 19 °C. If the life cycle of soil microbial nitrifiers is a duration of only several hours (Prosser 1986), this result is not surprising.

For the early spring samples (March 1996), the NO_3^--N concentrations in soils of TR2 ranged from 1.2 to 8.10 mg kg^{-1} (Table 1) and were mostly 0.8-1.88 times (Fig. 1) those of TR1 soils (0.3-6.60 mg kg^{-1}) with higher concentrations than those of TR1 soils in the

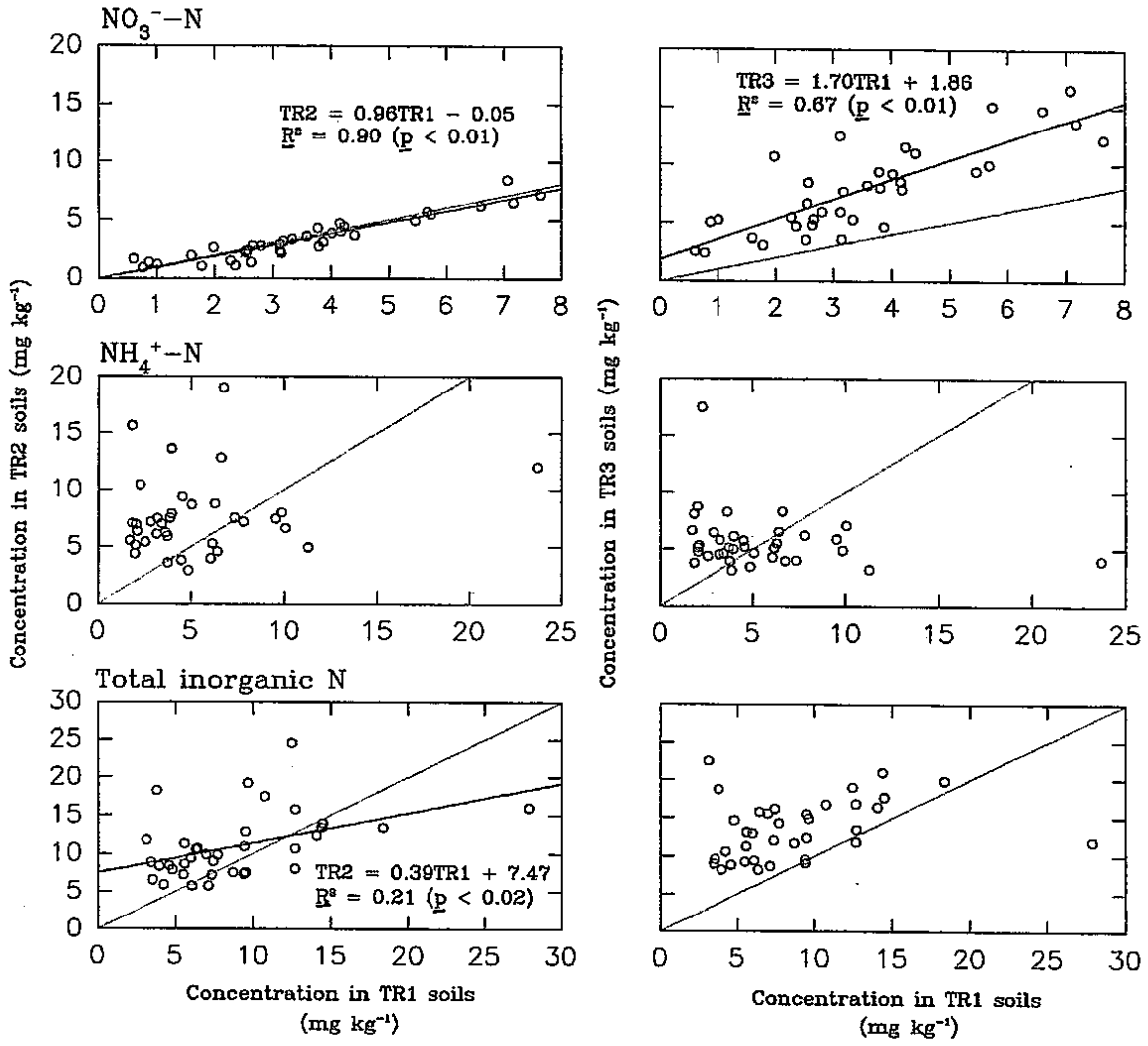

Fig. 2. Comparison of NO_3^--N, NH_4^+-N, and total inorganic N concentrations for soils between *in situ* extractions (TR1) and extracts soaked then extracted in the laboratory (TR2); and *in situ* extractions and extracts stored overnight then extracted in the laboratory (TR3) in July 1995. Dotted lines in the figure represent a 1:1 ratio of concentrations being compared, while the regression lines indicate trends of concentrations of TR2 or TR3 soils in relation to those of TR1 soils.

higher concentration range of TR1 soils (Fig. 3). The result was relatively similar to summer samples. NH_4^+ -N concentrations in soils of TR2 ranged from 6.50 to 23.20 mg kg^{-1} and were mostly around 0.83-1.80 times those in TR1 soils (5.10-20.60 mg kg^{-1}). The ratio range was far narrower than that in summer samples. For total inorganic available N, concentrations in the soils of TR2 ranged from 7.80 to 27.40 mg kg^{-1} and were around 0.9-2.0 times those of TR1 soils; and the ratio range was also narrower than those in summer samples. As to TR3 soils, NO_3^- -N concentrations were still higher than those of TR1 soils as in summer. However, the concentration range of TR3 soils was mostly only about 0.8-

2.5 times those of TR1 soils on this sampling date, which was far less than that in summer. The same situation also occurred for NH_4^+-N (1.0-3.5 times) and total available inorganic N (1.0-3.2 times) for soils of TR3 (Fig. 1). As a consequence, it seems that ammonification was the only factor that was greatly enhanced to increase the concentrations of NH_4^+-N in soils of TR3, which in turn increased concentrations of total available N in those soils. However, no particular trend was shown with the increase of NH_4^+-N concentrations in TR3 soils in relation to TR 1 soils (Fig. 3). Since soil moisture is normally not a constraining factor for soil microbial activity in the Fushan area, the lower temperature might be the factor greatly holding back soil nitrification and causing far smaller increases of NO_3^--N

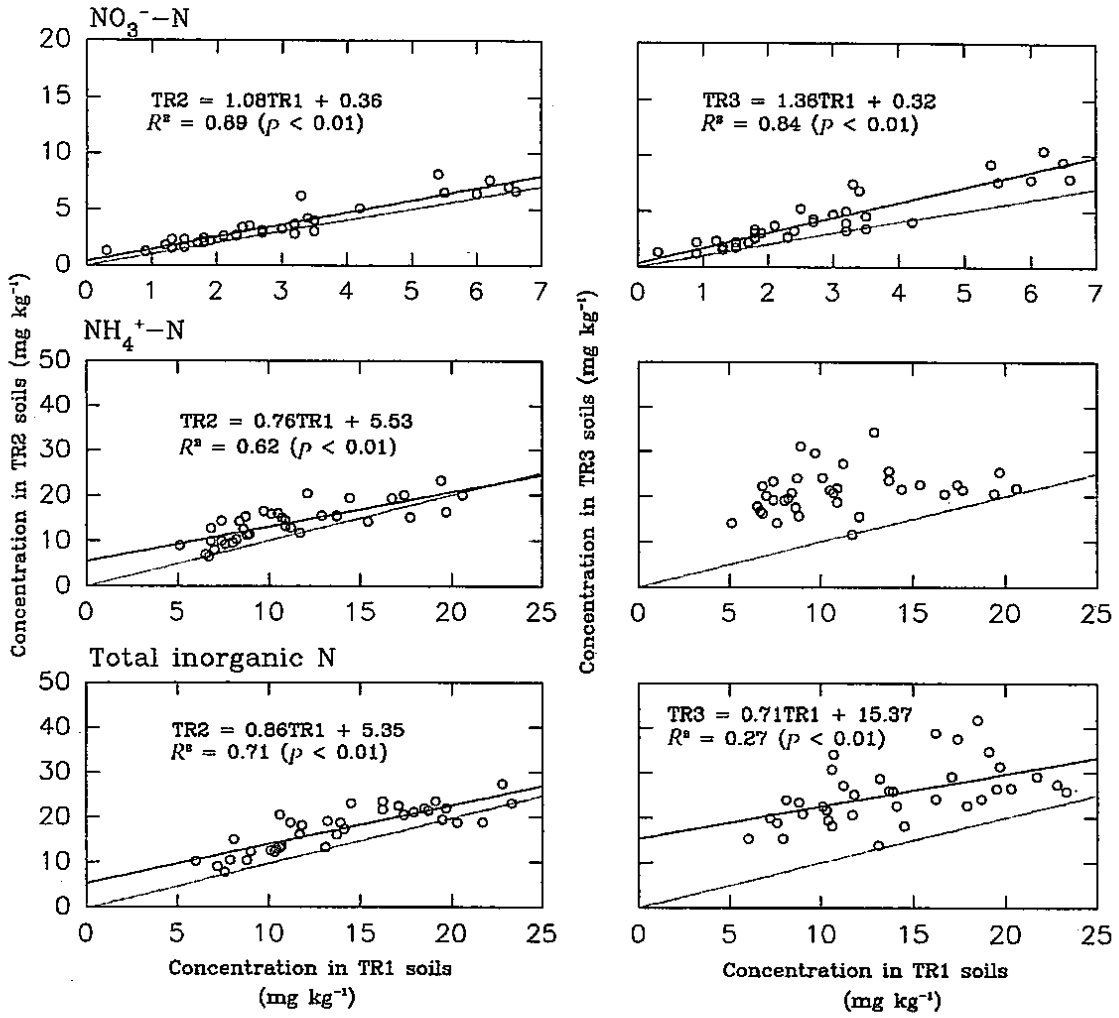

Fig. 3. Comparison of NO_3^--N, NH_4^+-N, and total inorganic N concentrations for soils between *in situ* extractions (TR1) and extracts soaked then extracted in the laboratory (TR2); and *in situ* extractions and extracts stored overnight then extracted in the laboratory (TR3) in March 1996. Dotted lines in the figure represent a 1:1 ratio of concentrations being compared, while the regression lines indicate trends of concentrations of TR2 or TR3 soils in relation to those of TR1 soils.

concentrations in soils of TR2 and TR3. However, at a highly fertile site in the Great Smoky Mountains National Park, North Carolina, USA, delay in soil processing only caused an increase of net nitrification, and no increase of net mineralization or ammonification in all seasons (van Miegroet 1995). It is apparent that the situation in subtropical or tropical regions is far more complicated.

Although some biocides can be applied to inhibit soil microbial activities during storage and transportation of soil samples, the release of inorganic nitrogen from dead soil microbes may also affect N concentrations (Tsai and Cresser 1998). From the above results, the immediate extraction of soil samples with KCl solution (TR1) is the only method which can obtain a good estimation of *in situ* available NO_3^--N, NH_4^+-N, and total inorganic N in soil. Any delay of extraction might cause one to overestimate or underestimate the N concentrations by allowing further ammonification, nitrification, or immobilization mediated by soil microbes. The situation is more serious in the warm summer than in the cool winter or early spring. If no simple extraction equipment is available in the field, it is better to keep soil samples in the extraction solution and to send them back quickly to the laboratory for extraction (TR2). In this way, one can obtain N estimations which are closer to those in the field than those of samples staying overnight (TR3), then being transported back and extracted in the laboratory.

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