Published by Avanti Publishers

Global Journal of Agricultural Innovation,
Research & Development

ISSN (online): 2409-5826

Global Journal of Agricultural Innovation, Research and Development
ISSN (online) 2409-9813

Optimizing Conditions for the Measurement of Soil Inorganic Nitrogen with a Micro-Plate Reader

Qihong Tu[1,2,*], Fugen Dou[2] and Haytham M. Salem[1,3,*]

[1] *Key Laboratory of Digital Land and Resources, East China University of Technology, Nanchang, Jiangxi 330013, China*
[2] *Texas A&M AgriLife Research Center, 1509 Aggie Drive, Beaumont, Tx 77713, USA*
[3] *Department of Soil and Water Conservation, Desert Research Center, 11753 Cairo, Egypt*

ARTICLE INFO

Article Type: Research Article

Keywords:
Nitrate N
Extraction
Ammonium N
Well microplate

Timeline:
Received: March 22, 2021
Accepted: May 06, 2021
Published: May 21, 2021

Citation: Tu Q, Dou F, Salem HM. Optimizing Conditions for the Measurement of Soil Inorganic Nitrogen with a Micro-Plate Reader. Glob J Agric Innov Res Dev. 2021; 8; 22-31

DOI: https://doi.org/10.15377/2409-9813.2021.08.2

ABSTRACT

There are numerous methods for measuring inorganic nitrogen (N) in soils, but many of these are complex or require expensive equipment. In order to seek an accurate and rapid measurement method, we optimized the conditions for soil inorganic nitrogen (N) (NH4+-N and NO3--N) determination with a micro-plate reader; a systematic study was carried out. The effects of extraction regent concentrations, ratios of extraction solution to the soil, extraction time, extracted solution storage methods and time, and reaction time on soil inorganic N measurement were investigated. Results showed that all tested conditions except the storage method had a significant effect on the determination of soil NH4+-N and NO3--N. Compared with the non-stored treatment, the value of the measured soil nitrate-nitrogen increased after being stored in a refrigerator for seven weeks, while that for ammonium-nitrogen content was reduced. The appropriate protocol was using extracting solution directly to determine the content of NH4+-N and NO3--N in soil (otherwise, keep it at room temperature), 1.0 mol/L KCl as the extraction regent, solution/soil ratio with 10:1, extraction for 45 min, and reaction for 25 min (only for NH4+-N). The recovery rate of adding standard solution was above 99% as it met the detection requirements.

---

*Corresponding Authors
Email: qihtu@163.com, eng_haytham1982@yahoo.com
Tel.: 86 13732909329, 00201142252406

1. Introduction

Nitrogen (N) is one of the most important crucial nutrients needed to achieve yield potentials for most crops and a critical component in soil fertility management, plant growth and crop production depend to a large extent on soil N-supplying capacity [1]. Both ammonium nitrogen and nitrate nitrogen are available nitrogen sources for plants and microorganisms, which can be directly taken up and utilized by crops [2]. The loss of nitrogen from fertilizers and crop residues in agricultural production into the water body is an important source of non-point pollution [3]. The content of ammonium nitrogen and nitrate nitrogen are important indicators of soil nutrient and environmental assessment. Therefore, accurate and fast determination of soil inorganic nitrogen has gradually played an important role in effective soil N management [4].

Chemical and biological methods are two main types of methods for determining soil mineral nitrogen that is widely used and promising in the world. The latter is complex and difficult to apply widely in practice, whereas the chemical method was developed based on biological methods, which is simple and reflects the actual nitrogen supply capacity in soil. As early as 1959, Livens proposed a simple method for extracting mineral nitrogen from the soil with boiling water [5]. At present, there are two commonly used chemical methods. One is the distillation method or the micro-diffusion method. However, because this method is purely manual measurement, it is not only time-consuming and laborious but also the measurement process is easily interfered with by many factors, leading to the results with significant variations. The other is measured by an instrument, which mainly includes phenol disulfonic acid colorimetry, ion electrode method and chromatography, ultraviolet spectrophotometry, reflectometer method, diffusion-conductivity method, or continuous flow analysis method[6-13]. All these methods are difficult to meet the conventional batch measurement requirements due to the following drawbacks [14-15]: (1) tedious operation steps; (2) long time of measurement; (3) expensive equipment and high operating skill requirements; and (4) unstable value with poor precision. In addition, inorganic nitrogen results measured by continuous flow analysis method often have negative values, which affects the accuracy of the measurement results, and seriously affects plant nutrition and soil workers' understanding and research process of available nitrogen nutrition in the soil. Ultraviolet spectrophotometry does not require tedious operations, and the price of the instrument is relatively low. Some progress has been made in the determination of inorganic nitrogen, and its reliability has been proven. Compared with the above-mentioned methods, a microplate method is more rapid and simpler and needs less consumables [5, 16-20]. Reaction, color development, and absorbance determination were all able to be completed in a single 96-well. The method extracting with 2 mol/L boiled KCl for 4 hours and with 1 mol/L boiled KCl for 1 hour were successively proposed by Gianello and Bremner [22]. At present, in addition to KCl, K_2SO_4 and $CaCl_2$ are also used as extraction reagents for soil inorganic nitrogen. The extraction method by regent was mostly adopted for the determination of soil inorganic nitrogen content [23-26]. KCl was generally considered the most common extraction agent for soil inorganic nitrogen [27-31]. It is good to increase crop yield to determine the NH_4^+-N and NO_3^--N in soil accurately and rapidly.

Different extractants have different effects on the determination of inorganic nitrogen. Nitrogen is usually extracted with reagents such as distilled water or diluted salt solution. However, the extraction by distilled water will cause soil clay particles to disperse, making it difficult to obtain clear solutions for analysis [32]. Compared with extraction by $CaCl_2$ and K_2SO_4, extraction solutions by KCl show relatively good recovery efficiency, especially suitable for simultaneous determination of ammonium and nitrate nitrogen [33]. Storage of extracted solution at different temperatures had no significant effect on NH_4^+-N and NO_3^--N concentration or the NH_4^+-N concentration varied greatly with storage temperature. The storage time of extracted solution can significantly affect the determination of inorganic nitrogen [34-35].

Although much research on the influencing factors on soil inorganic nitrogen measurement was carried out in recent years [36-37], including different concentrations of extraction regent and ratio of solution to the soil, but such effects on the analysis conducted by a microplate method have not been well determined. Therefore, in order to determine the NH_4^+-N and NO_3^--N in soil accurately and rapidly, the objective of this study is to standardize and improve effective extraction methods of soil mineral nitrogen, improve the accuracy of soil mineral nitrogen determination, and provide a basis for optimizing inorganic nitrogen measurement technology, which would be beneficial for N management in crop production.

2. Materials and Methods

2.1. Soil Samples

Two soil samples were used for this experiment, one clay soil from Texas A&M AgriLife Research and Extension Center near Beaumont, Texas, USA, and the other from the Research Station near Eagle Lake, Texas. The soil at Beaumont was a League clay soil (fine, montmorillonite, Entic Pelludert) with 28% silt, 58% clay, 1.2% organic matter, and pH 5.5. The soil at Eagle Lake was a Hockley silt loam (fine, smectitic, hyperthermic Typic Albaqualfs) with 19% silt, 15% clay, 0.7% organic matter, and pH 5.9. The cropping systems for both sites have been one-year rice and two-year fallow for decades. The soil was sampled before plowing at a depth of 0-20 cm in April 2018. Soil samples were air-dried, passed through a 2 mm sieve, and 4 g of soil sample were weighed and put into a 50-ml centrifuge tube. After adding 16 mL of 2.0 mol/L KCl solution, the tubes were shaken for 0.5 h at 25 °C and centrifuged at 6,000 r/min for 5 min with a centrifuge (Corning LSEC) and filtered by a filter paper (Fisher brand). Futhermore, the filtrate was prepared for the determination of NH_4^+-N and NO_3^-N concentration by a microplate reader (BioTek Epoch-12). Each treatment had three replicates. Basic physical and chemical properties are provided in Table **1**.

Table 1: Properties of the Soil Samples

Soil	Ammonia N (mg/kg)	Nitrate N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	pH	Ec (umho/cm)
BMT	9.48	2	47	96	5.6	125
EL	7.35	41	76	224	5.4	125

BMT-Beaumont organic soil; EL-Eagle Lake soil.

2.2. Experimental Design and Methods

2.2.1. Extracting Solution and Concentration

Soil samples passed through a 2-mm sieve were extracted with 16 ml of deionized water (the control), 0.01 mol/L, 0.5 mol/L, 1.0 mol/L, 1.5 mol/L, 2.0 mol/L, and 2.5 mol/L KCl for NH_4^+-N and NO_3^-N concentration, respectively.

2.2.2. Ratio of Extracting Solution to Soil

Extraction reagents with different ratios of extraction solution-to-soil [4:1, 6:1, 8:1and 10:1 (volume: mass)] were used to determine the effects on NH_4^+-N and NO_3^-N extraction.

2.2.3. Extraction Time

Three times (30, 45, and 60 min) were designed to shake at 25 °C to determine the adequate duration for the extraction.

2.2.4. Storage Methods and Storage Time at Different Temperature

Soil samples extracted with 2.0 mol/L KCl were prepared to determine the NH_4^+-N and NO_3^-N concentration. The extracting solutions were divided into four equal portions. A was preserved at room temperature; B was preserved in a refrigerator at 4 °C; C was preserved in a freezer at - 19 °C; D was determined immediately. The four equal portions were stored for 0 d, 3 d, 7 d, 15 d, 30 d, and 50 d at their respective temperatures.

2.2.5. Reaction Time for NH4+-N Concentration

Different reaction times of 2 min, 5 min, 8 min, 10 min, 15 min, 20 min, 25 min, 30 min, 35 min, and 40 min were designed to determine the ammonium N.

2.3. Determination of Ammonium Nitrogen and Nitrate Nitrogen by a Microplate Reader

2.3.1. Determination of Ammonium N Using a 96-Well Plate

First, 80 ul buffer solution was added into each well, followed by 30 ul extracted sample solution; then 60 ul sodium salicylate was added and mixed well by shaking the plate shaker. Finally, 90 ul NaOCl solution was added into each well, followed by shaking the plate again, and then the plate was put in the dark to react for 30 min. After that, the plate was read by a PowerWave plate-reader at 660 nm, according to Stephanie *et al*. [11].

2.3.2. Determination of Nitrate N Using a 96-Well Plate

The specific procedure for nitrate N determination was used as follows:

(1) rinsed a nitrate reduction redactor with DI water and acid cleaning solution; (2) placed it in a holding tray containing pH 8.5 1.0% NH_4Cl buffer before use; (3) pipetted 20 ul aliquots of standard solutions and samples into each well of a clean microplate; (4) pipetted 200 ul pH 8.5 1.0% NH4Cl buffer into each well containing samples and standard solutions; (5) placed the microplate secured the redactor with locking pins on a shaker for 60 min; (6) after the shaking, added 60 ul of the mixed Griess reagent to the microplate containing the samples and standard solutions; as well as, placed it on the shaker for 15 min to complete the color development; (7) determined the optical densities of the plate on the microplate reader at 542 nm, according to Stephanie *et al*. [11].

2.4. Statistical Analysis

Statistical evaluation of the data was performed by Microsoft Excel 2013 and SPSS Statistics 25.0 (SPSS Armonk, NY: IBM Corp; 2019), and the least significant difference (LSD 0.05) was used for multiple comparisons of NH_4^+-N and NO_3^-‾N concentrations using the effects of different factors. Data are shown as mean ± standard error (n=3).

3. Result and Discussion

3.1. Effect of Soil Extraction Reagent Concentrations on the Determination of Ammonium N and Nitrate N

For ammonium N, the results showed that there was a significant difference ($P < 0.05$) in the measured N between DI water and any concentration of KCl solution extracts (Table **2**). Compared to DI water, any KCl extract had a greater measured N concentration.

Table 2: Effect of Different Leaching Solution Concentrations on the Determination of NH_4^+-N (mg/kg)

Soil	DI Water	Different KCl Solution Concentration (mol/L)					
		0.01	0.5	1.0	1.5	2.0	2.5
BMT	3.67 ± 0.41^c	5.83 ± 0.98^b	7.01 ± 0.24^b	8.91 ± 0.86^a	9.02 ± 0.39^a	9.48 ± 0.55^a	8.93 ± 0.80^a
EL	3.52 ± 0.30^c	5.57 ± 0.31^b	6.20 ± 0.06^{ab}	7.09 ± 0.58^a	7.38 ± 0.61^a	7.35 ± 0.19^a	6.89 ± 0.40^a

Different letters in the same line mean significant (P<0.05) difference. The value showed as means and standard deviations. BMT- Beaumont organic soil; EL- Eagle Lake soil; DI Water- Distilled Water.

As in Table **3**, the results showed that there was no significant difference between the DI water extraction and the low concentration KCl solution until the concentration reached 0.5 mol/L. Smith and Li (1993) revealed that the increase of KCl concentrations from 1 mol/L to 2 mol/L had no significant effect, while the variations were small when the concentration of KCl was increased to 3 mol/L [21]. In our study, for the BMT soil, the nitrate N was increasing when the concentration increased from 0.5 mol/L to 2.5 mol/L, but the difference was not significant ($P < 0.05$), indicating the 0.5 mol/L KCl solution was able to fully extract the nitrate-nitrogen. While for the EL soil, the nitrate N showed a downward trend when the concentration increased from 0.5 mol/L to 2.5mol/L, and the content of nitrate N reached the maximum when the concentration was 1.0 mol/L KCl.

Table 3: Effect of Different Leaching Solution Concentrations on the Determination of NO_3^--N (mg/kg)

Soil	DI Water	Different KCl Solution Concentration (mol/L)					
		0.01	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**
BMT	1.30±0.16^{c}	1.48±0.22^{b}	2.01±0.93^{a}	1.96±0.42^{a}	2.15±0.14^{a}	2.25±0.11^{a}	2.28±0.05^{a}
EL	25.46±0.45^{d}	25.69±0.11^{d}	29.28±0.39^{ab}	29.58±0.32^{a}	27.71±0.09^{bc}	28.38±0.39^{b}	27.26±0.22^{c}

Different letters in the same line mean significant (P<0.05) difference. The value are showed as means and standard deviations. BMT- Beaumont organic soil; EL- Eagle Lake soil; DI Water- Distilled Water.

Based on the results of Table **2** and **3**, we concluded that the ammonium N and nitrate N could fully be extracted by 1.0 mol/L KCl for two soil samples selected in our study. The results are the same with Lei [36].

3.2. Effect of Different Ratios of Extraction Solution to Soil on the Determination of Ammonium N and Nitrate N

The results indicated that as the ratio of extraction solution to soil increased, the content of ammonium nitrogen showed an increasing trend, and when the ratio of solution to soil reached 10:1, the content of ammonium nitrogen reached the max, compared with other treatments. While for the content of nitrate nitrogen, there was no significant difference ($P < 0.05$) between the treatments except for BMT soil with 4:1 extraction, indicating that the ratios of extraction solution to soil had minimal effect on the content of nitrate-nitrogen in the soil tested. So we concluded that the ratio of 10:1 would be better, which is consistent with the theory of adsorption and desorption.

Table 4: Effect of Different Ratio of Solution to Soil on the Determination of NH_4^+-N and NO_3^--N (mg/kg)

Ratio of Agent to soil	NH_4^+-N Content (mg/kg)		NO_3^--N Content (mg/kg)	
	BMT Soil	**EL Soil**	**BMT Soil**	**EL Soil**
4:1	10.40±0.40^{c}	7.46±0.29^{c}	1.59±0.19^{b}	24.87±0.29^{a}
6:1	12.35±0.14^{b}	8.90±0.09^{b}	2.01±0.05^{a}	24.88±0.29^{a}
8:1	13.06±0.10^{b}	9.48±0.30^{b}	2.11±0.09^{a}	25.19±0.03^{a}
10:1	15.70±0.25^{a}	11.36±0.25^{a}	2.38±0.09^{a}	25.13±0.21^{a}

Different letters in the same line mean significant (P<0.05) difference. The values are showed as means and standard deviations. BMT Soil- Beaumont organic soil; EL Soil- Eagle Lake soil.

3.3. Effect of Different Extraction Times on the Determination of Ammonium N and Nitrate N

Both ammonium N and nitrate N responded differently to the different shaking times (Table **5**). Ammonium N increased with increasing shaking time but with a faster rate from 30 to 45 minutes than from 45 to 60 minutes. However, nitrate N did not respond to an increase in shaking time. Zhang *et al*. [37] studied the effects of different extraction times from 10 to 50 minutes on the determination of soil nitrate N, and the results showed that the value reached greater after 20 minutes and no significant changes with the extension of time. Li *et al*. [32] found

Table 5: Effect of Different Times of Shaking on the Determination of NH_4^+-N and NO_3^--N (mg/kg)

Shaking Time (min)	NH_4^+-N Content (mg/kg)		NO_3^--N Content (mg/kg)	
	BMT Soil	**EL Soil**	**BMT Soil**	**EL Soil**
30	7.53^{b}	6.18^{b}	2.27^{a}	26.12^{a}
45	14.21^{a}	12.33a	2.35^{a}	26.36^{a}
60	15.51^{a}	12.87^{a}	2.22^{a}	25.98^{a}

Different letters in the same line mean significant (P<0.05) difference. The value showed as means and standard deviations. BMT Soil- Beaumont organic soil; EL Soil- Eagle Lake soil.

that compared to 30 and 120 min, shaking for 60 min gave greater results for soil NO_3^--N in most cases, but ammonium-N was not significantly affected by shaking time. It is inconsistent with our results, and the reason for the different results may be related to different soil types. Another reason is the different time range, our study on extraction time from 30 to 60 minutes.

3.4. Effect of Different Storage Methods and Storage Time of Soil Extraction Solution on the Determination of Ammonium N and Nitrate N

From Table **6** to **9**, the results showed that there was no significance ($P < 0.05$) between different storage methods, but the value of the soil extraction solution stored in the refrigerator was larger than that stored at room temperature; no matter what kind of storage method, there is no significant difference ($P < 0.05$) between the direct determination treatment and the treatments of after 7 days or 19 days storage, but a significant difference ($P < 0.05$) was seen after 30 days of storage. Therefore, they can be safely stored directly at room temperature within 30 days when no immediate measurement is available. In addition, NH_4^+-N content became gradually smaller with time extension, whereas there was an increasing trend for NO_3^--N content, indicating a possible nitrification transformation. However, they were smaller than the values of treatments of direct measurement after extraction. Therefore, it is better to determine the content of inorganic nitrogen immediately when the soil is extracted.

Table 6: Effect of Different Preservation Methods and Preservation Time of Soil Extraction Solution on the Determination of NH_4^+-N (mg/kg)

Different Treatments	**NH_4^+-N Content of Different Preservation Time (mg/kg)**				
	0d(DS1)	**7d**	**19d**	**30d**	**50d**
AS1	14.88±0.74^{a}	13.46±0.34^{ab}	13.02±0.16^{ab}	11.48±0.99^{b}	11.15±0.58^{b}
BS1	14.88±0.74^{a}	14.17±1.16^{ab}	13.82±0.34^{ab}	12.30±0.07^{b}	12.19±0.43^{b}
CS1	14.88±0.74^{a}	13.86±0.27^{ab}	13.20±0.29^{ab}	12.74±0.92^{b}	11.58±0.54^{b}
AS2	10.32±0.14^{a}	9.91±0.15^{a}	9.94±0.16^{a}	7.20±0.67^{b}	6.99±0.14^{b}
BS2	10.32±0.14^{a}	9.85±0.23^{ab}	9.67±0.11^{b}	8.46±0.08^{c}	8.18±0.24^{c}
CS2	10.32±0.14^{a}	9.82±0.07^{ab}	9.69±0.16^{b}	7.85±0.15^{c}	7.61±0.24^{c}

Different letters in the same line mean significant (P<0.05) difference. The value showed as means and standard deviations. A- Stored at room temperature; B- Stored at 4 ° C; C- Stored at -19 ° C; S1-BMT organic soil; S2- Eagle Lake soil.

Table 7: Effect of Different Preservation Methods and Preservation Time of Soil Extraction Solution on the Determination of NO_3^--N (mg/kg)

Different Treatments	**NO_3^--N Content of Different Preservation Time (mg/kg)**				
	0d(DS1)	**7d**	**19d**	**30d**	**50d**
AS1	2.11±0.05^{b}	2.23±0.19^{ab}	2.08±0.06^{b}	2.36±0.11^{ab}	2.53±0.04^{a}
BS1	2.11±0.05^{c}	2.17±0.06^{c}	2.18±0.10^{c}	2.41±0.06^{b}	2.69±0.05^{a}
CS1	2.11±0.05^{b}	2.19±0.09^{b}	2.25±0.06^{b}	2.30±0.08^{b}	2.60±0.06^{a}
AS2	23.63±0.61^{b}	23.20±0.36^{b}	23.48±0.02^{b}	25.45±0.08^{a}	26.53±0.56^{a}
BS2	23.63±0.61^{c}	23.63±0.20^{c}	24.37±0.27^{bc}	25.34±0.16^{b}	27.18±0.32^{a}
CS2	23.63±0.61^{c}	23.53±0.35^{c}	23.96±0.36^{c}	25.48±0.45^{b}	26.91±0.30^{a}

Different letters in the same line mean significant (P<0.05) difference. The values are showed as means and standard deviations. A- Stored at room temperature; B- Stored at 4 ° C; C- Stored at -19 ° C; S1-BMT organic soil; S2- Eagle Lake soil.

Table 8: Effect of Different Preservation Methods and Preservation Time of Soil Extraction Solution on the Determination of NH_4^+-N (mg/kg)

Preservation Time (Day)		Different Preservation Methods		
		A	B	C
S1	7	13.46±0.34^{a}	14.17±1.16^{a}	13.86±0.27^{a}
	19	13.02±0.16^{a}	13.82±0.34^{a}	13.20±0.29^{a}
	30	11.48±0.99^{a}	12.30±0.07^{a}	12.74±0.92^{a}
	50	11.15±0.58^{a}	12.19±0.43^{a}	11.58±0.54^{a}
S2	7	9.91±0.15^{a}	9.85±0.23^{a}	9.82±0.07^{a}
	19	9.94±0.16^{a}	9.67±0.11^{a}	9.69±0.16^{a}
	30	7.20±0.67^{a}	8.46±0.08^{a}	7.85±0.15^{a}
	50	6.99±0.14^{b}	8.18±0.24^{a}	7.61±0.24^{ab}

Different letters in the same line mean significant ($P<0.05$) difference. The values are showed as means and standard deviations. S1-BMT organic soil; S2- Eagle Lake soil.

Table 9: Effect of Different Preservation Methods and Preservation Time of Soil Extraction Solution on the Determination of NO_3^--N (mg/kg)

Preservation Time (Day)		Different Preservation Methods		
		A	B	C
S1	7	2.23±0.19^{a}	2.17±0.06^{a}	2.19±0.09^{a}
	19	2.08±0.06^{a}	2.18±0.10^{a}	2.25±0.06^{a}
	30	2.36±0.11^{a}	2.41±0.06^{a}	2.30±0.08^{a}
	50	2.53±0.04^{a}	2.69±0.05^{a}	2.60±0.06^{a}
S2	7	23.20±0.36^{a}	23.63±0.20^{a}	23.53±0.35^{a}
	19	23.48±0.02^{a}	24.37±0.27^{a}	23.96±0.36^{a}
	30	25.45±0.08^{a}	25.34±0.16^{a}	25.48±0.45^{a}
	50	26.53±0.56^{a}	27.18±0.32^{a}	26.91±0.30^{a}

Different letters in the same line mean significant ($P<0.05$) difference. The values are showed as means and standard deviations. S1-BMT organic soil; S2- Eagle Lake soil.

3.5. Effect of Different Reaction Times on the Determination of Ammonium N

The OD was measured by indophenol blue colorimetric within 2-40 minutes. From Figure **1**, the results showed that the OD became larger as the reaction time prolonged, and it was stable after 25 minutes. There was no significant difference between 25 to 40 minutes treatments. Therefore, to sufficiently complete the colorimetric reaction, the minimum time is 25 minutes or longer.

3.6. Recovery Test for Adding the Standard Solution

Recovery test for adding standard solution was carried out under the above optimal condition, including 1.0 mol/L KCl used as the extraction regent, ratio of solution to soil with 10:1, the extraction time for 45 minutes, and the ammonium nitrogen determination reaction time for 25 minutes. The different gradient concentrations of $(NH_4)_2SO_4$ standard solution were added to the samples to determine the recovery rate of added ammonium nitrogen content in the two kinds of soil. The results (Table **10**) showed that the recovery rate was above 94%, and the accuracy of the method can meet the detection requirements. It demonstrated that the quantitative recovery of $(NH_4)_2SO_4$ is possible by this method.

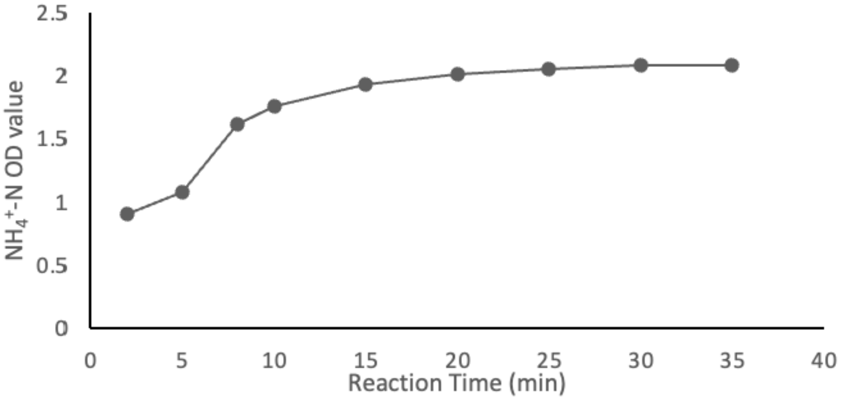

Figure 1: The OD curve of ammonium nitrogen with different reaction time

Table 10: Recovery Tests of the Method

Standard Samples	Added (ug)	Found (ug)	Recovery (%)
CK	0	0.67±0.01	
C_1S	0.25	0.90±0.01	94.84
C_2S	0.50	1.14±0.02	94.84
C_3S	0.75	1.43±0.04	101.50
C_4S	1.00	1.70±0.80	103.58
C_5S	1.25	2.01±0.07	107.82

Values showed as means and standard deviations. S- Beamont organic soil. C1, C2, C3, C4 and C5 means 5, 10, 15, 20, 25 ppm Standard solution, respectively.

4. Conclusions

In this study, we concluded that the conditions optimized for the determination of ammonium nitrogen and nitrate-nitrogen in the soil are 1.0 mol/L KCl as the extraction regent, solution/soil ratio with 10:1, extraction for 45 min, and reaction for 25 min (just for ammonium nitrogen). The findings also suggest that immediate determination or storage of soil extract at room temperature within 30 days could improve the reliability of NH_4^+-N and NO_3^--N results.

The recovery rate was above 94%, and the accuracy of the method can meet the detection requirements. The microplate spectrophotometric method is with good precision, high efficiency, and less waste production, within 90 min for nitrate and 30 min for ammonium, and was successfully applied to actual soil sample analysis with a recovery of 94%.

Acknowledgments

The authors thank the Jiangxi Province Key Lab for Digital Land (DLLJ201807) for financial supporting and the Science and Technology Plan Project of Jiangxi Provincial Department (GJJ170448).

References

[1] Li, S. X., Wang, Z. H., Malhi, S. S., Li, S. Q., Gao, Y. J., and Tian, X. H. 2009. Nutrient and water management effects on crop production, and nutrient and water use efficiency in dryland areas of China. Advances in Agronomy 102:221–262. Doi:10.1016/S0065-2113(09)01007-4.

[2] Zhu, Z. L., Wen, Q. X. 1994. Soil nitrogen of China. Jiangsu Science and Technology Press 1-303.

[3] Zhu, Z. L., Chen, D. L. 2002. Nitrogen fertilizer use in China-Contributions to food production, impacts on the environment and best management strategies. Nutrient Cycling in Agroecosystems 63(2):117-127. doi:10.1023/A:1021107026067.

[4] Harmsen, G. W., and Van Schreven, D. A. 1955. Mineralization of organic nitrogen in soil. Advances in Agronomy 7:299-398. doi:10.1016/S0065-2113(08)60341-7.

[5] Livens, J. 1959. Contribution à l'étude de l'azote minéralisable du sol. Agr. Louvain 7: 27-44.

[6] Carlson, R. M. 1978. Automated separation and conductimetric determination of ammonia and carbon dioxide. Analytical Chemistry 50:1528-31. doi:10.1021/ac50033a035.

[7] Carlson, R. M. 1986. Continuous flow reduction of nitrate to ammonia by granular zinc. Analytical Chemistry 50:1590-91. doi:10.1021/ac00298a077.

[8] Saha, U. K., L. Sonon., and D. Kissel. 2012. Comparison of conductimetric and colorimetric methods with distillation-Titration method of analyzing ammonium nitrogen in total kjeldahl digests. Communications in Soil Science and Plant Analysis 43:2323-41. doi:10.1080/00103624.2012.708081.

[9] Uttam K. Saha, Leticia Sonon., and Bipul K. Biswas. 2018. A Comparison of Diffusion-Conductimetric and Distillation-Titration Methods in Analyzing Ammonium- and Nitrate-Nitrogen in the KCl-Extracts of Georgia Soils. Communications in Soil Science and Plant Analysis. 49:1, 63-75, doi: 10.1080/00103624.2017.1421647.

[10] Dou, H., A. K. Alva., and T. Appel. 2000. An evaluation of plant-available soil N in selected sandy soils by electro-ultrafiltration, KCl, and CaCl2 extraction methods. Biology and Fertility of Soils. 30:328–332. doi:10.1007/s003740050011.

[11] Stephanie, R., Lara, S., and Zackary I. J. 2011. A suite of microplate reader-based colorimetric methods to quantify ammonium, nitrate, orthophosphate and silicate concentrations for aquatic nutrient monitoring. J. Environ. Monit. 13:370–376. doi: 10.1039/c0em00290a.

[12] Tu, X. H., Xiao, B. D., Xiong, J., and Chen, X. D. 2010. A simple miniaturised photometrical method for rapid determination of nitrate and nitrite in freshwater. Talanta 82: 976–983. doi:10.1016/j.talanta.2010.06.002.

[13] Bremner, J. M., and Keeney, D. R. 1965. Steam distillation method for determination of ammonium, nitrite, and nitrate. Analytica Chimica Acta 32:485-95. doi:10.1016/S0003-2670(00)88973-4.

[14] James, D., and John, H. G. 2011. A new cadmium reduction device for the microplate determination of nitrate in water, soil, plant tissue, and physiological fluids. Journal of AOAC International 94(6): 1896-1905. doi:10.5740/jaoacint.10-454.

[15] Ye, X. S., and Zhao, Z. Q. 2011. Comparison between flow injection analyzer method and national standard method in determining soil nitrate-N concentration. Hubei Agricultural Science. 50(4): 698-707.

[16] D'Angelo, E., Crutchfield, J., and Vandiviere, M. 2001. Rapid, sensitive, microscale determination of phosphate in water and soil. Journal of Environmental Quality 30(6): 2206-2209. doi:10.2134/jeq2001.2206.

[17] Wu, J. P., Hong, Y. G., Guan, F. J., Wang, Y., Tan, Y. H., Yue, W. Z., Wu, M. L., Bin, L. Y., Wang, J. P., Wen, J. L. 2016. A rapid and high-throughput microplate spectrophotometric method for field measurement of nitrate in seawater and freshwater. Scientific reports, 6:1-9. doi: 10.1038/srep20165.

[18] Wang, Y. H., Salatasa, A., Minesa, P. D., Jakobsenc, M. H., and Andersen, H. R. 2016. Graduated characterization method using a multi-well microplate forreducing reactivity of nanoscale zero valent iron materials. Applied Catalysis B: Environmental 181:314–320. doi: 10.1016/j.apcatb.2015.07.041.

[19] Rebecca, H. N., Nina, H. N. U., Erich, I., Petra, O. L., Wolfgang, W. 2010. Alternative Methods for Measuring Inorganic, Organic, and Total Dissolved Nitrogen in Soil. Nutrient Management & Soil & Plant Analysis. 74(3): 1018-1027. Doi: 10.2136/sssaj2009.0389.

[20] Crutchfield, J. D., Grove, J. H. 2011. A New Cadmium Reduction Device for the Microplate Determination of Nitrate in Water, Soil, Plant Tissue, and Physiological Fluids. Journal of AOAC International. 94(6): 1896–1905. doi: 10.5740/jaoacint.10-454.

[21] Smith, K. A., and Li, S. X. 1993. Estimation of potentially mineralisable N in soil by KCl extraction. Plant and Soil 157:167–174. doi: 10.1007/BF00011046.

[22] Gianello, C., and Bremner, J. M. 1986. A simple chemical method of assessing potentially available organic nitrogen in soil. Communications in Soil Science and Plant Analysis 17:195-214. doi:10.1080/00103628609367708.

[23] Westfall, D. G., Henson, M.A., Evans, E. P. 1978. The effect of soil sample handling between collection and drying on nitrate concentration. Communications in Soil Science and Plant Analysis 9(2):169-185. doi:10.1080/00103627809366797.

[24] Lin, S., Miihling, K. H., Sattelmacher, B. 1997. Soil nitrogen fraction as influenced by sample preparation and extraction. Communications in Soil Science and Plant Analysis 28(6):551-559. doi:10.1080/00103629709369810.

[25] Vaughan, B., J. Denning., and H. Frank. 1995. Bray and Kurts phosphorous (PI), 1M potassium chloride, and saturated calcium oxide extraction of soil nitrate comparison to 2M potassium chloride. Communications in Soil Science and Plant Analysis 26:453-57. doi:10.1080/00103629509369310.

[26] Houba, V. J. G., Temminghoff, E. J. M., Gaikhorst, G. A., and Vark, W. van. 2008. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Communications in Soil Science and Plant Analysis. 31(9): 1299-1396. doi: 10.1080/00103620009370514.

[27] Kachurina, O. M., H. Zhang, W. R. Raun., and E. G. Krenzer. 2000. Simultaneous determination of soil aluminum, ammonium- and nitrate-nitrogen using 1M potassium chloride extraction. Communications in Soil Science and Plant Analysis 31:893-903. doi:10.1080/00103620009370485.

[28] Zhao, R. F., Yu, Z. Y., Cheng, B., Zhang, Q. 2009. Effects of pretreatment on content of soil NO3--N, NH4+-N. Chinese Agricultural Science Bulletin 25(10):174-177.

[29] McTaggart, I. P., Smith, K.A. 1993. Estimation of potentially mineralisable nitrogen in soil by KCl extraction. Plant and Soil 157:175-184. doi:10.1007/BF00011046.

[30] Murphy, D. V., Macdonald, A. J., Stockdale, E. A, Goulding, K. W. T., Fortune, S., Gaunt, J. L., Poulton, P. R., Wakefield, J. A., Webster, C. P., and Wilmer, W. S. 2000. Soluble organic nitrogen in agricultural soils. Biology and Fertility of Soils 30(5/6): 374-387. doi:10.1007/s003740050018.

[31] Kachurina, O. M., Zhang, H., Raun, W. R., and Krenzer, E. G. 2000. Simultaneous determination of soil aluminum, ammonium- and nitrate-nitrogen using 1M potassium chloride extraction. Communications in Soil Science and Plant Analysis 31: 893-903. doi:10.1080/00103620009370485.

[32] Li, K. Y., Zhao, Y. Y., Yuan, X. L., Zhao, H. B., Wang, Z. H., Li, S. X., and Malhi, S. S. 2012. Comparison of factors affecting soil nitrate nitrogen and ammonium nitrogen extraction. Communications in Soil Science and Plant Analysis 43: 571-588. doi:10.1080/00103624.2012.639108.

[33] Tao, S. U., Ru, S. M., Wang, Z. H., Li, S. X. 2005. Effects of pretreatment, shaking and conserving method and extracting solution on results for soil mineral nitrogen. Journal of Agro-Environment Science 24(6): 1238-1242.

[34] Houba, V. J. G., Temminghoff, E. J. M., Gaikhorst, G. A. 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction regent. Communications in Soil Science and Plant Analysis 31:1299-1396. doi:10.1080/00103620009370514.

[35] Ma, B. L., Ying, J., Balchin, D. 2005. Impact of sample preservation methods on the extraction of inorganic nitrogen by potassium chloride. Journal of Plant Nutrition 28(5):785-796. doi:10.1081/PLN-200055536.

[36] Lei, W. Q., Zhang, Z. X., Zhang, H. M., Wang, J. L., Ren, X. S. 2019. Comparative research on determination of ammonium nitrogen in soil which extracted by potassium chloride and sodium chloride. Forum of South China 43-44. doi:10.1080/00103624.2012.639108.

[37] Zhang, L.,Yang, S.S.,Cao, B.J.,Hu, B.,Peng. H.W., Chen, X.,Liu, S.B.,Liu, Y.D.,Zhu, Y. 2018. Optimization of nitrate-N extraction technology from Tobacco planting soil. Guizhou Agricultural Sciences. 46(2): 66-69.