Improved Method for Extracting Nitrites in Soil

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Abstract: Soil nitrite (NO_2^-) is an important reactive intermediate in many nitrogen transformation processes, but it is unstable under acidic conditions and may be lost as gaseous N. The canonical extraction method of soil NO_2^- using a potassium chloride (KCl) solution greatly underestimates its concentration. To reflect the concentration more accurately, we optimized the extraction method of soil NO_2^- for three agricultural soils differing in soil texture and pH, an alkalic fluvo-aquic soil and acidic Mollisol and Ultisol soils, respectively. Both extractable soil ammonium (NH_4^+) and nitrate (NO_3^-) were systematically investigated to optimize the simultaneous extraction of soil inorganic nitrogen. The effects of different extractants (deionized water (DIW), un-buffered 2 mol L^{-1} KCl, and pH-buffered 2 mol L^{-1} KCl), shaking time (10 and 30 min), and storage duration of the extracts (stored at −20 °C for 1 day, and at 4 °C for 1, 3, and 6 days) on the determination of soil inorganic nitrogen were investigated. The results showed that the un-buffered KCl extractant significantly underestimated soil NO_2^- concentration compared to DIW. The highest recovery of NO_2^- was obtained by extracting with DIW at 10 min of shaking for all three soils. Compared with DIW, the concentration of NH_4^+ and NO_3^- in soil extracted from the KCl solution increased significantly. Furthermore, the soil inorganic nitrogen content of extracts stored at 4 °C for one day was closer to the direct measurements of fresh samples than with the other storage methods. Overall, the recommended analysis method for soil NO_2^- was extraction by DIW, shaking for 10 min, and filtering with a 0.45 µm filter, while soil NH_4^+ and NO_3^- were extracted with a KCl solution and shaken for 30 min. The extract should be stored at 4 °C and analyzed within 24 h. Our study provides an efficient extraction method for soil NO_2^- and supports studies on the biogeochemical nitrogen cycle, e.g., in the investigation of soil nitrous acid (HONO) and nitric oxide (NO) emissions.

Keywords: soil nitrites; deionized water; potassium chloride; shaking time; storage time

1. Introduction

Nitrite (NO_2^-) plays a crucial role in the soil nitrogen cycle as a key intermediate in several nitrogen transformation processes (nitrification, denitrification, nitrifier denitrification,

dissimilatory nitrate reduction to ammonium (DNRA), and chemo-denitrification) [1–6] and is also closely associated with gaseous nitrogen emissions as an independently existing nitrogen reservoir [7]. For example, NO_2^- is directly associated with NO and N_2O gas formation. When $NO_2^- < 1$ mg N kg^{-1}, it still promotes NO production [8]. NO_2^- is also an important precursor for N_2O formation, and more than 44% of N_2O changes are associated with NO_2^- [9]. Due to its extremely rapid turnover, it is considered an instantaneous product and has often been neglected in previous soil nitrogen (N) cycle studies [10–12]. However, NO_2^- accumulation occurs when some environmental factors lead to the decoupling of ammonia oxidation from nitrite oxidation [13,14]. For example, the application of urea or ammonium-based fertilizers may cause instantaneous accumulation of NO_2^- during nitrification [9,10,15], which is exacerbated at higher soil pH [16]. Soil NO_2^- accumulation may cause a number of environmental problems; for example, NO_2^- reacts with other substances to form gases such as nitrous acid (HONO), nitric oxide (NO), nitrogen dioxide (NO_2), and nitrous oxide (N_2O). This affects the oxidizing capacity of the atmosphere, air quality, and human health, and it can also act as a strong greenhouse gas [17–19]. The importance of NO_2^- in water and soil for gaseous N emissions has been increasingly acknowledged. Accurate and rapid determination of soil NO_2^- is therefore needed to achieve a deeper understanding of N transformation processes in soil.

Typically, a 2 mol L^{-1} KCl solution is used to extract NO_2^- from soil, which is also a classical method for simultaneously extracting soil ammonium (NH_4^+) and nitrate (NO_3^-) [20]. During the extraction of NO_2^-, there are generally no biological or chemical reactions that cause changes in its concentration or isotopic composition [21]. However, KCl causes NO_2^- degradation under acidic and neutral conditions, greatly underestimating the NO_2^- concentration in soil [16,20]. Studies have shown that only 22–40% of NO_2^- is recovered from soils with a pH between 4.8–5.4 [20,22]. Nevertheless, the extraction of soil NO_2^- with a 2 mol L^{-1} KCl solution is still a recommended method to be used in soil analysis manuals and international standards [23–25].

Previously, new methods have been proposed to improve the extraction efficiency of NO_2^- in soil. For instance, the recovery of NO_2^- in acidic soil was higher when extracted by deionized water (DIW) from a small amount (4 g) of soil, but the suspended solids in the DIW extracts affected the absorbance measurements during spectrophotometric NO_2^- analysis [20]. To maximize the recovery of soil NO_2^-, the pH of the extraction solution with calcium hydroxide [Ca(OH)$_2$] [26], calcium carbonate ($CaCO_3$) [27], or potassium hydroxide (KOH) [21] was adjusted. Stevens and Laughlin (1995) [21] adjusted the pH of the extraction solution to 8.0 with 2 mol L^{-1} KOH and a soil/extractant ratio of 1:1, which resulted in a significant increase in NO_2^- recovery. However, this method is only applicable to a substantial amount of soil (200 g) [20], and deviation from this parameter may lead to dispersion of silt and clay particles and/or the dissolution of organic matter [28], making it difficult to obtain a clear solution for analysis [20]. This adjustment process is cumbersome and increases the experimental time and effort. Due to the strong buffering effect of acidic soils, it is difficult to keep the pH of the adjusted KCl extract stable, and the pH may still decrease after oscillation, which may lead to the loss of NO_2^-. In addition, a higher pH tends to cause volatilization losses of NH_3 [29], potentially decreasing NH_4^+ recovery if the same extraction method is applied.

In recent years, a large number of studies have been conducted on the factors affecting the determination of soil NH_4^+ and NO_3^-, including the extraction method [30], the concentration of the extractant [30], the storage method [31], the shaking time [30], etc. However, the effects of these factors on soil NO_2^- concentration have not been systematically reported. Therefore, this study aims to improve the effective extraction and accuracy of soil NO_2^- determination, and to provide a basis for realizing the efficient extraction of NO_2^-, NO_3^-, and NH_4^+.

2. Materials and Methods

2.1. Soil Sampling

The soils used here (fluvo-aquic soil, Mollisol, and Ultisol) were previously studied by Song et al. (2023) [19] and have been described in detail. Briefly, the fluvo-aquic soil was collected from Quzhou County, in the North China Plain (36°52′ N, 115°10′ E), with a pH of 7.98, soil organic matter (SOM) of 17.59 g kg^{-1}, total nitrogen (TN) of 0.11%, and C/N of 9.23, respectively. The soil clay, silt, and sand fractions were 0.65%, 84.47%, and 11.88%, respectively. The soil net nitrification rate and the net mineralization rate were 16.92 and 19.55 mg kg^{-1} day^{-1}, respectively. The climate is a temperate monsoon climate, with an average annual temperature and precipitation of 13.2 °C and 494 mm, respectively. The crops planted are a rotation of wheat and maize. The Mollisol was collected from Lishu County in Northeast China (43°37′ N, 124°36′ E). It has a pH of 5.51, SOM of 22.76 g kg^{-1}, TN of 0.13%, and C/N of 10.13, respectively. The soil clay, silt, and sand fractions were 3.85%, 75.62%, and 20.53%, respectively. The soil net nitrification rate and net mineralization rate were 15.36 and 21.45 mg kg^{-1} day^{-1}, respectively. The climate is a temperate monsoon climate, with an average annual temperature and precipitation of 6.5 °C and 650 mm, respectively. The planted crop is maize. The Ultisol was collected from Jinjing City in Central South China (28°38′ N, 113°19′ E). It has a pH of 5.35, SOM of 18.45 g kg^{-1}, TN of 0.11%, and C/N of 9.72, respectively. The soil clay, silt, and sand fractions were 0.07%, 77.49%, and 22.44%, respectively. The soil net nitrification rate and net mineralization rate were 11.05 and 11.05 mg kg^{-1} day^{-1}, respectively. The climate is a subtropical monsoon climate, with an average annual temperature and precipitation of 17.5 °C and 1330 mm, respectively. The crops planted are oilseed rape and maize. Five sampling points were randomly selected on each site, and samples were taken at a depth of 20 cm before combining them into one composite sample. After removing roots, rocks, seeds, and other debris, the soil was air-dried. After grinding, the soil was sieved through a 2 mm sieve and then kept at room temperature.

2.2. Experimental Design

We first compared the effect of different extraction methods on inorganic N concentrations (DIW, un-buffered 2 mol L^{-1} KCl, and pH-buffered 2 mol L^{-1} KCl) to assess whether increasing the pH in the extraction solution would affect the extractable NO_2^-. Three groups of treatments were set up: (1) only DIW; (2) un-buffered 2 mol L^{-1} KCl; (3) pH = 7.5, 6.5, and 6.0 phosphate buffer solutions (PBSs) + 2 mol L^{-1} KCl (1:4) were added to the fluvo-aquic soil; and pH = 8.4, 8.0, 7.5, and 7.0 phosphate buffer solutions + 2 mol L^{-1} KCl (1:4) were added to the Mollisol and Ultisol samples, respectively. Different pH values of PBS were prepared by mixing 1/15 mol L^{-1} KH_2PO_4 and Na_2HPO_4 in different proportions. Air-dried soils (5 g; $n = 3$; sieved to 2 mm) were extracted in 25 mL DIW, un-buffered 2 mol L^{-1} KCl, or pH-buffered 2 mol L^{-1} KCl (the soil-liquid ratio of all extractions was 1:5 (weight/volume, w/v)), followed by shaking at 200 rpm and 25 °C for 30 min, centrifugation at 9000 rpm for 15 min, and filtration, first through 9 cm medium speed filter paper (aperture 15–20 μm) and then through 0.45 μm aperture filter paper. The inorganic nitrogen content of the extracting solution was measured by a SmartChem automatic chemical analyzer 450 (AMS-Alliance, Rome, Italy). The pH of the extracting solution was measured by a pH meter (Mettler Toledo FE28, Schwerzenbach, Switzerland) and mixed at a 1:2.5 soil to water ratio (w/v).

We further investigated the effect of storing the extracts for variable time periods. Briefly, air-dried soils (5 g, $n = 3$) were extracted in 25 mL un-buffered 2 mol L^{-1} KCl or DIW and mixed at a 1:5 soil-liquid ratio (w/v), followed by shaking at 200 rpm and 25 °C for 30 min, centrifugation at 9000 rpm for 15 min, and filtration, first with 9 cm medium speed filter paper (aperture 15–20 μm) and then with 0.45 μm aperture filter paper. Aliquots of the extracts were taken for direct determination of soil inorganic nitrogen concentration, while the remainder was stored at −20 °C for 1 day and at 4 °C for 1, 3, and 6 days, respectively, before analysis.

Finally, we studied the effect of shaking duration on soil NO_2^- recovery. NO_2^- standard solution was added to evaluate the recovery of NO_2^- in the extracts. The background value of NO_2^- is low in acidic soils, and in order to be able to accurately detect changes in NO_2^- content, the amount of NO_2^- added in this experiment was 3 mg N kg^{-1} soil. Soil samples were extracted with DIW and pH-buffered 2 mol L^{-1} KCl. The extraction method was as described above. The difference was that shaking was 10 min and 30 min, respectively, and the extract was placed at 4 °C for storage and analyzed within 24 h. All the above treatments were set up in triplicates. Soil without the addition of standard solution was used as a control. The recovery of NO_2^- was calculated based on the difference measured in soil with and without adding the standard solution:

$$Recovery = \frac{N_b - N_a}{N_o} \times 100\% \tag{1}$$

where N_b and N_a represent the NO_2^- content of soil with added standard solution and control soil, respectively; N_o represents the NO_2^- concentration in the standard solution.

2.3. Statistical Analyses

All test data were preliminarily sorted and in-depth calculated by Microsoft Excel 2019. SPSS Statistics 26.0 (IBM, Armonk, NY, USA) was used for a one-way ANOVA or a two-factor ANOVA to determine the differences in extractable soil inorganic nitrogen between treatments (*t* and LSD test, $p < 0.05$). When necessary, data were transformed to meet the assumption of normality.

3. Results

3.1. Higher Soil NO_2^- Contents with DIW Extraction

The pH values of the un-buffered KCl extracts were 7.68, 4.16, and 4.65 for the fluvo-aquic soil, Mollisol, and Ultisol, respectively. These values were significantly lower than the pH of the DIW extracts (Figure 1). Adding a phosphate buffer solution decreased the pH of the extracts significantly in the fluvo-aquic soil, while it increased the pH in the Mollisol and Ultisol samples. The pH values were 7.14, 6.17, and 5.96 for the pH-buffered KCl extracts in thefluvo-aquic soil, while they were maintained at pH 6.0–7.5 in Mollisol and Ultisol.
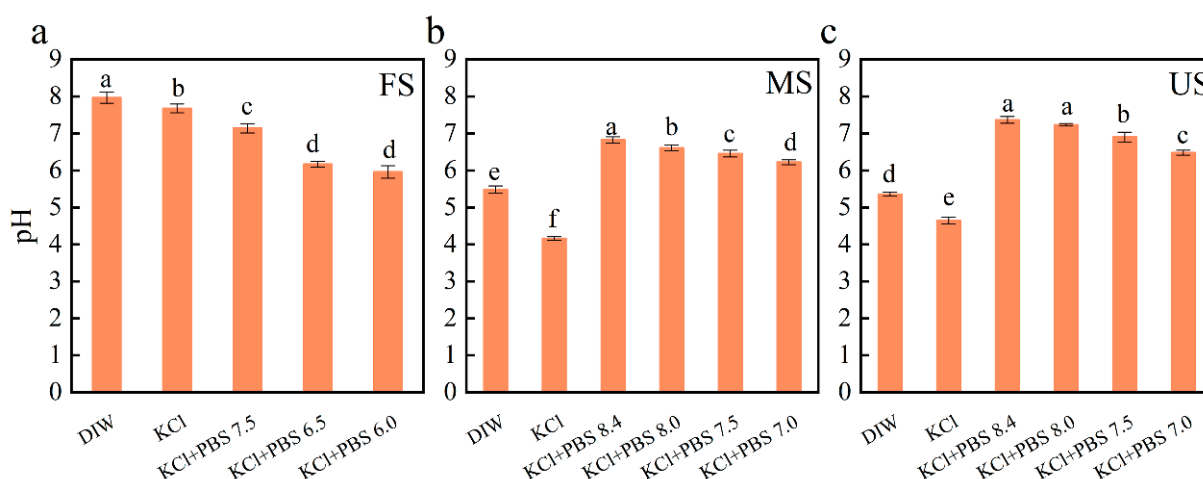

Figure 1. Variation in pH of soil extracts from different treatments. (**a**) FS; (**b**) MS; (**c**) US. DIW, deionized water; KCl, 2 mol L^{-1} KCl; PBS 8.4, 8.0, 7.5, 7.0, 6.5, and 6.0 denote phosphate buffer solutions with pH values of 8.4, 8.0, 7.5, 7.0, 6.5, and 6.0, respectively. FS: fluvo-aquic soil; MS: Mollisol; US: Ultisol. Different lowercase letters indicate significant differences at $p < 0.05$.

The variation of inorganic nitrogen in different treatments is shown in Figure 2. The NO_2^- content was significantly higher in the fluvo-aquic soil (1.18–2.06 mg N kg^{-1}) than in Mollisol (0.02–0.03 mg N kg^{-1}) and Ultisol (0.02–0.08 mg N kg^{-1}) ($p < 0.05$, Figure S1). Extraction with DIW resulted in a higher NO_2^- content as compared with the un-buffered KCl solution in all three soils. Extractable NO_2^- increased when using a phosphate buffer solution with different pH but was still lower than that in the DIW extractions. However, soil NH_4^+ concentration was reduced by 25% (fluvo-aquic soil), 35% (Mollisol), and 60% (Ultisol) when extracted by DIW compared with the un-buffered KCl treatment, respectively. The soil NO_3^- concentrations were significantly higher in the un-buffered KCl treatment than in the DIW extracts of fluvo-aquic soils, while the opposite was true for Mollisol and Ultisol. It was further found that the NH_4^+ and NO_3^- content of Ultisol was significantly higher than that of the other two soils in the un-buffered KCl treatment ($p < 0.05$, Figure S1b).
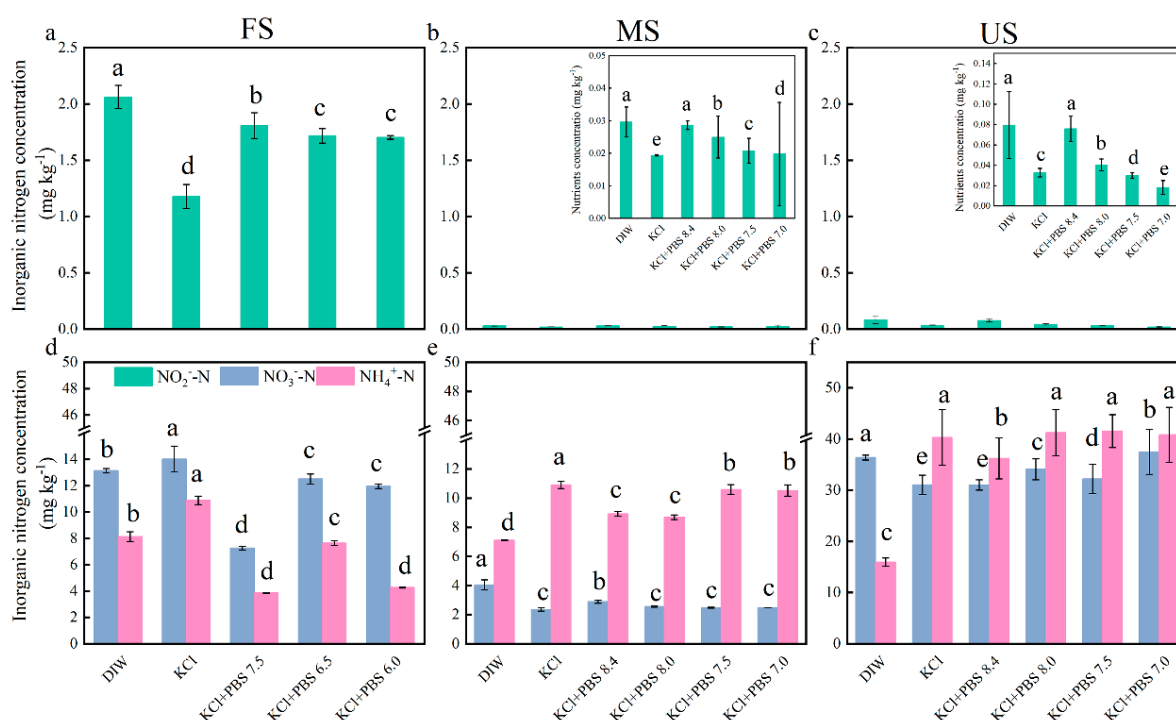

Figure 2. Variation in inorganic nitrogen concentration in different treated soils. (**a–c**) are FS, MS, and US NO_2^- concentrations, and (**d–f**) are FS, MS, and US NH_4^+ and NO_3^- concentrations, respectively. DIW, deionized water; KCl, 2 mol L^{-1} KCl; PBS 8.4, 8.0, 7.5, 7.0, 6.5, and 6.0 denote phosphate buffer solutions with pH values of 8.4, 8.0, 7.5, 7.0, 6.5, and 6.0, respectively; FS: fluvo-aquic soil; MS: Mollisol; US: Ultisol. Different lowercase letters indicate a significant difference between treatments for the same species of inorganic nitrogen ($p < 0.05$).

3.2. Storage Increased NO_2^- and NH_4^+ Content

Storage significantly affected the concentration of inorganic nitrogen in the extracts (Figure 3). When calculating the soil NO_2^- content, it was closer to the directly measured content of fresh samples when stored at 4 °C than at −20 °C, regardless of extraction with DIW or un-buffered KCl treatment. There was a tendency for DIW extracts to increase with longer storage time at 4 °C ($p < 0.05$). Compared to storage for 1 d, storage for 6 d increased the soil NO_2^- content by 3.4 (fluvo-aquic soil), 1.6 (Mollisol), and 1.8 folds (Ultisol), respectively. The soil NO_3^- concentration extracted by un-buffered KCl treatment decreased with the storage time (4 °C), while the opposite was the case for NH_4^+. The soil NH_4^+ concentration increased by 51% (fluvo-aquic soil), 59% (Mollisol), and 20% (Ultisol) after storing the extracts for 6 d compared to 1 d. We found no significant difference

between storing for 1 d at 4 °C and directly measuring DIW-extracted NO_2^- and un-buffered KCl-extracted NH_4^+ concentrations ($p > 0.05$). Further studies revealed that the inorganic nitrogen concentration differed significantly among the three soils ($p > 0.05$, Figure S2).
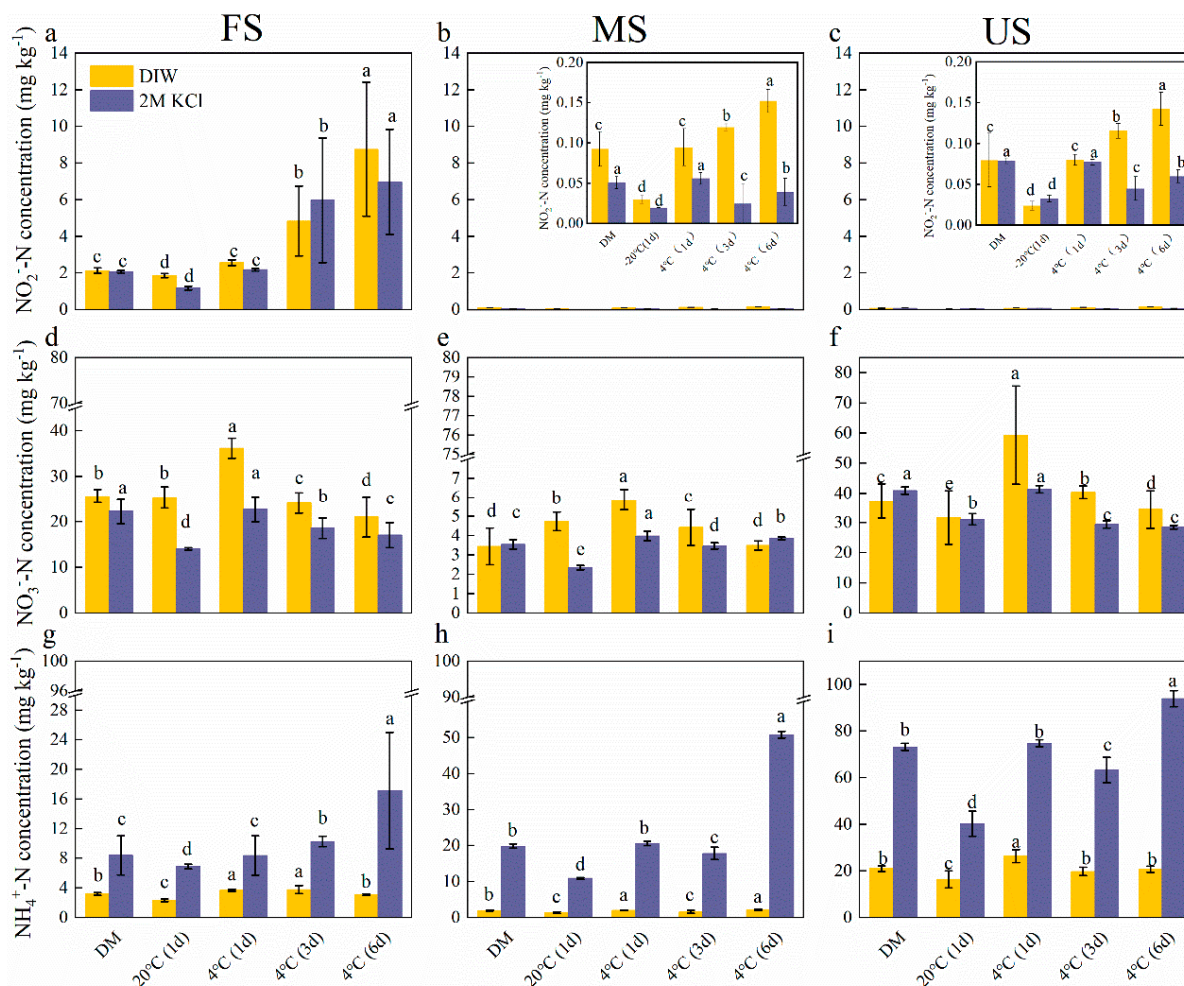

Figure 3. Changes in inorganic nitrogen concentration in extracts from different storage methods. (**a–c**) are the concentrations of FS, MS, and US NO_2^-, (**d–f**) are the concentrations of FS, MS, and US NO_3^-, (**g–i**) are the concentrations of FS, MS, and US NH_4^+, respectively. DM, directly measured; −20 °C (1d), stored at −20 °C for 1 day; 4 °C (1d), (3d), and (6d) means that the samples were stored at 4 °C for 1, 3, and 6 days, respectively; FS: fluvo-aquic soil; MS: Mollisol; US: Ultisol. Different lowercase letters indicate a significant difference between treatments for the same soil extract ($p < 0.05$).

3.3. Effect of Shaking Time on NO_2^- Recovery

Shaking time had a significant effect on NO_2^- recovery in acidic soils (Mollisol and Ultisol) but not in the alkaline soil (fluvo-aquic soil) (Figure 4). When shaking for 10 min, the NO_2^- recovery rates of the three soils were 85–87% (fluvo-aquic soil), 92–95% (Mollisol), and 91.39–91.72% (Ultisol), respectively, for both extraction methods (DIW and pH-buffered KCl). No significant difference was found among the treatments ($p > 0.05$). With the increase of shaking time, the recovery of NO_2^- in acidic soil decreased, especially in the DIW treatment. The NO_2^- recovery in acidic soils was affected by the pH of the extractant. After shaking for 30 min, the NO_2^- recovery rates of the pH-buffered treatments were 88% (Mollisol) and 90% (Ultisol), respectively, which were significantly higher than those of the DIW treatments ($p < 0.05$). The results of a two-way ANOVA showed that there was a

significant interaction effect between pH and shaking time on the recovery of NO_2^- in the acidic soils, but not in the alkaline soil ($p > 0.05$). In summary, extraction with DIW and shaking for 10 min could be suitable for both acidic and alkaline soils.
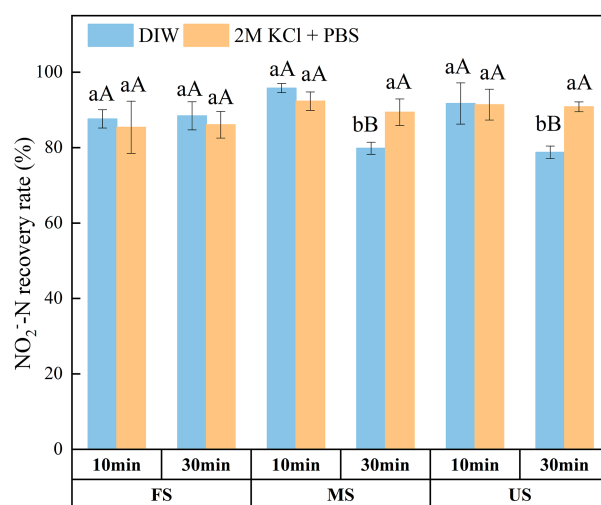

Figure 4. Soil NO_2^- recovery rate with different oscillation time. DIW, deionized water; 2 M KCl + PBS, 2 mol L^{-1} KCl, and PBS with different pH values: pH 7.5 of PBS is added to the fluvo-aquic soil, and pH 8.4 of PBS is added to Mollisol and Ultisol; FS: fluvo-aquic soil; MS: Mollisol; US: Ultisol. Different lowercase letters indicate a significant difference between the DIW and KCl + PBS treatments at $p < 0.05$. Different uppercase letters indicate a significant difference between different oscillation times at $p < 0.05$.

4. Discussion

4.1. Soil NO_2^- Concentration

Compared with DIW, extracting with an un-buffered KCl solution significantly reduced the pH of extracts in Mollisol and Ultisol (Figure 1b,c), which may be due to the release of protons by K$^+$ adsorption, an increase in ionic strength, and the hydrolysis of extractable aluminum. In acidic environments, NO_2^- protonates into HNO_2 (pK$_a$ = 3.3, 25 °C), which subsequently decomposes to gaseous nitrogen products such as HONO, NO, or NO_2, a process that can explain the loss of NO_2^- in acidic environments [19,20,32]. These results were confirmed by lower NO_2^- concentrations (35–59%) in the un-buffered KCl extract compared to those extracted by DIW (Figure 2a–c). In addition, the chemical fixation of NO_2^- by insoluble soil organic matter [22,33], the reaction with metal oxides [34], or the rapid conversion to NO_3^- [27,33] may also be responsible for the low NO_2^- concentration in the un-buffered KCl extract. To maximize the recovery of soil NO_2^-, the pH of the extracting solution was adjusted with Ca(OH)$_2$ [26], CaCO$_3$ [27], or KOH [21]. Indeed, the pH of the pH-buffered KCl extracts increased significantly (Figure 1b,c), and the NO_2^- contents also increased significantly (Figure 2b,c). The extracted NO_2^- by the pH 8.4 buffer solution was even comparable to that of DIW in Mollisol and Ultisol. It was found that the recovery of NO_2^- was about 69% when the extract's pH was between 4.8 and 6.0, and it increased to as much as 95% when the pH was adjusted to 7.8 [34]. In conclusion, extraction by un-buffered KCl could greatly underestimate soil NO_2^- concentrations.

Although more NO_2^- can be extracted with DIW, it produces a brown extract that interferes with colorimetric analysis (Figure 5). Homyak et al. (2015) [20] found that the concentration of NO_2^- in DIW extract measured by colorimetric analysis was 35% higher than that determined by ion chromatography. However, conventional filters did not remove suspended particles, and the color reagent flocculated with the suspended solids, resulting in higher absorbance readings and an overestimation of the NO_2^- concentration. The measured results were consistent after filtration through a 0.45 μm filter [20].
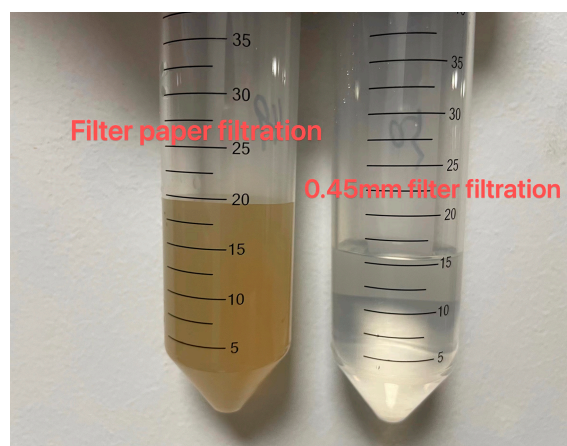
Figure 5. Soil NO_2^- was extracted with deionized water to produce a brown extract, which was filtered through a 0.45 μm filter to obtain a clear extract.

Shaking time was also a key factor for soil NO_2^- recovery. NO_2^- recovery from acidic soil decreased significantly with an increase in shaking time (Figure 4). Stevens and Laughlin (1995) [21] showed that the NO_2^- recovery after 10 min of shaking was 86%, which was three times higher than that after 30 min of shaking. Our study showed that the NO_2^- recovery from acidic Mollisol and Ultisol extracted by DIW was greater than 90% at 10 min of shaking. The NO_2^- recovery was higher when extracting for 30 min at a higher pH.

During the analysis process, the extracts may not be determined immediately and may be stored for a period of time because of logistical reasons. However, few studies have considered the effect of the storage duration on soil NO_2^- concentration. We found that the NO_2^- concentration of samples stored at 4 °C was closer to the directly measured values of fresh samples, whereas freezing to −20 °C resulted in severe losses of NO_2^- (Figure 3a–c). The reason may be due to NO_2^- degradation caused by freezing and rapid NO_2^- turnover during thawing [35]. There were also significant differences in NO_2^- concentration among different soils ($p < 0.05$, Figure S2). In addition, the NO_2^- concentration gradually increased with the increase in storage time (4 °C), likely due to nitrification in the non-sterile extract [35]. Therefore, it is recommended that NO_2^- should be measured as soon as possible after extraction to obtain more accurate value.

4.2. Soil NH_4^+ and NO_3^- Concentrations

Compared with un-buffered KCl, extraction with DIW significantly underestimated the NH_4^+ concentration in the three soils and the NO_3^- concentration in the fluvo-aquic soil (Figure 2d–f). Tu et al. (2021) [30] also found that the measured concentrations of NH_4^+ and NO_3^- in KCl extracts were 1.6–2.6 and 1.1–1.8 folds higher than those in DIW, respectively. Lim et al. (2018) [16] showed that DIW-extracted NO_3^- concentrations were only 50–60% of those extracted by 2 mol L^{-1} KCl. Due to the significant ion exchange capacity of soil [16], soil colloids are usually negatively charged and can adsorb NH_4^+, while high concentrations of K^+ can displace NH_4^+ adsorbed on soil colloids. However, the negatively charged NO_3^- ions will not be adsorbed by the soil and will "freely" exist in soil solution.

Although most of the literature suggests that the shaking time for extracting NH_4^+ and NO_3^- should be 60 min [30,36], some studies believe that the concentration of NO_3^- does not change significantly after 15–30 min of shaking [37,38]. The NH_4^+ and NO_3^- concentrations were relatively stable at the late stage of shaking (30–60 min), indicating that 30 min of shaking is long enough to quantitatively extract soil NH_4^+ and NO_3^- [38]. For soils with very high mineral nitrogen content, the shaking time may be extended to 60 min. In this study, NH_4^+ and NO_3^- were extracted with a shaking time of 30 min.

The storage conditions of the extracts significantly affected the soil NH_4^+ and NO_3^- contents. We found that the soil NH_4^+ or NO_3^- contents were close to the directly measured values of fresh samples when the un-buffered KCl or DIW extracts were stored at 4 °C. These values were also higher than those at −20 °C (Figure 3d–i). The NH_4^+ and NO_3^- contents in extracts stored at −20 °C were only 50–60% and 60–80% of those at 4 °C, respectively. This may be due to the degradation of NH_4^+ and NO_3^- due to the freezing or thawing process. A gradual increase in NH_4^+ and decrease in NO_3^- in soil extracts stored at 4 °C (Figure 3d–i) was also reported by Li et al. (2012) [36]. Thus, we recommend that the extracted solution be stored at 4 °C and analyzed within 24 h. In addition, we observed significant differences between the NH_4^+ and NO_3^- concentrations in Ultisol in the above treatments and other soils ($p < 0.05$, Figure S2), a phenomenon which suggests that the nature of the soil has an influence on the results of the study.

5. Conclusions

The soil NO_2^- extraction method was optimized in order to more accurately reflect the true concentration of NO_2^- in soil. Un-buffered KCl significantly underestimated soil NO_2^- concentration compared to DIW. Further studies showed that the shaking time and the storage method significantly affected the NO_2^- concentration. The highest NO_2^- recoveries for the three soil NO_2^- extracted by DIW were obtained at 10 min of shaking. The soil inorganic nitrogen content in extracts stored at 4 °C for one day was closer to the direct measurements of fresh samples than with other storage methods. Overall, the optimal conditions for determination of soil NO_2^- involved using DIW as the extractant, shaking for 10 min, and passing the solution through a 0.45 μm filter. Soil NH_4^+ and NO_3^- were extracted using conventional KCl solution. Both extracts should be measured immediately. Otherwise, they should be stored at 4 °C and analyzed within 24 h. Our study achieved the efficient extraction of soil NO_2^-, NO_3^-, and NH_4^+, respectively. Further optimization is needed if we want to achieve their efficient extraction at the same time.

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Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

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