Received: 3 November 2023 | Accepted: 13 February 2024

DOI: 10.1002/saj2.20653

Soil Science Society of America Journal

TECHNICAL NOTE

Agricultural Soil and Food Systems

Soil sample storage conditions affect measurements of pH, potassium, and nitrogen

Maya Sollen-Norrlin | **Naomi Laura Jane Rintoul-Hynes**

School of Natural and Applied Sciences, Canterbury Christ Church University, Canterbury, UK

Correspondence
Maya Sollen-Norrlin, School of Natural and Applied Sciences, Canterbury Christ Church University, Canterbury, CT1 1TE, UK.
Email: maya.sollennorrlin@canterbury.ac.uk

Assigned to Associate Editor Udayakumar Sekaran.

Funding information
Canterbury Christ Church University, Grant/Award Number: Graduate Scholarship Scheme

Abstract

Soil quality monitoring schemes are a useful tool for assessing the potential of soils to perform desired services such as agricultural productivity. When researchers or other stakeholders wish to compare results between different schemes or studies, failure to consider differences in soil sample storage conditions presents a significant potential for error. Here, we compared levels of nitrogen and potassium, as well as pH, in agricultural soil samples stored under three different conditions (refrigerated, frozen, and oven-dried). All tests were performed after 7 and 24 weeks of storage. Nitrate decreased significantly in dried ($p < 0.001$) samples. When refrigerated, nitrate first increased ($p < 0.01$) and then decreased ($p < 0.001$). Nitrate levels where unchanged at Week 7 in the freezer but decreased significantly at Week 24 ($p < 0.001$). Nitrite and ammonium increased after drying ($p < 0.001$) and when frozen ($p < 0.001$ and $p < 0.05$) but remained stable when refrigerated. There was no significant difference in potassium levels between the fresh control and Week 7 in the freezer, but potassium had increased at Week 24 ($p < 0.05$). Potassium concentration increased in refrigerated samples ($p < 0.001$) and fluctuated up and down in dried samples ($p < 0.01$). pH measurements fluctuated significantly in refrigerated and frozen samples ($p < 0.001$ and $p < 0.01$, respectively) but were unchanged in dried samples. We suggest that soil monitoring schemes standardize their sample storage, and we encourage researchers to clearly report soil sample storage conditions in publications, to improve transparency and reproducibility.

1 | INTRODUCTION

Soils are the primary sources of nutrients for human consumption (Huang et al., 2020) and an estimated 98% of calories originate in soils (Kopittke et al., 2019). Through complex interactions between living organisms and the physical and chemical properties of the soil environment, soils perform many ecosystem services (Pereira et al., 2018). These include food and feed production, nutrient cycling, water retention and filtration, carbon sequestration and climate change mitigation, and habitat provisioning (Delgado-Baquerizo et al., 2020).

Soil quality refers to the capacity of a soil to maintain these ecological functions and support a well-balanced ecosystem both below and aboveground (Sims et al., 1997).

Decades of unsustainable land-use practices, such as intensive use of pesticides, artificial irrigation, excessive tillage, and use of synthetic fertilizers, have led to increasing global

Abbreviations: LUCAS, land use and coverage area frame survey; SQI, soil quality indicator.

soil degradation (FAO et al., 2020). Up to 90% of the land area in Europe is considered to be degraded to some degree (Stavi & Lal, 2015) which impacts human and environmental health (Brevik et al., 2018).

Considering the fundamental role of soils in supporting environmental sustainability and human societies, soil monitoring schemes play a crucial role in our ability to continually assess soil health (Lehmann et al., 2020). For example, one focus in the United Kingdom's 25-Year Environment Plan is "using and managing land sustainably" (DEFRA & Rt Hon Gove, 2018). As part of the 25-year plan, the new Sustainable Farming Incentive is being implemented with a strong focus on improving soils, including the development of a soil health index building on the one used in the Countryside Survey (Emmet et al., 2010).

In the European Union (EU), the Land Use and Coverage Area Frame Survey (LUCAS) is used to obtain soil quality information from topsoil across the EU to assess the effects of land management on soil characteristics (Jones et al., 2013). Soil monitoring schemes provide the opportunity to assess the potential of soils to deliver ecosystem services under different land uses and over varying temporal scales, enabling the implementation of evidence-based policies for bringing about more sustainable land management (Haney et al., 2018). When assessing soil quality, it is important to consider the desired function of the soil under assessment and define soil quality in the context of its land use, for example, crop productivity and environmental sustainability are desired functions in agricultural soils (Oliver et al., 2013). The concept of soil quality indicators (SQIs) is based on the idea that some soil properties that are comparably easy to test and relatively sensitive to change, will represent the key functions of the soil and can act as indicators of overall soil quality (Bünemann et al., 2018). Andrews et al. (2004) define SQIs as "those soil properties and processes that have greatest sensitivity to changes in soil function" (p. 1945). While in situ testing of soils in the field might give the most accurate results (Bailey et al., 2021), it is not always feasible from a practical and logistical perspective. The alternative to in situ testing involves the removal of soil samples to the laboratory and the subsequent use of wet-chemistry techniques to analyze the soil physiochemical properties. This removal of soil from the field site creates the need for storing soil samples both during transport and in the laboratory before testing can commence. In the context of a soil monitoring scheme, it can take weeks or months to complete sampling across all sites, making sample removal and subsequent, often long-term, storage inevitable (Rutgers et al., 2009). Additionally, studies carried out by early career researchers such as postgraduate students often rely on a small number of people carrying out laboratory work, meaning that samples can require long-term storage simply because sample processing is done over a larger timescale. Since sample storage can affect measurements of soil properties (Barbagelata & Mallarino, 2013; Ma et al., 2005; Turner & Romero, 2009), it is important to standardize storage method whenever spatial or temporal comparisons are required. For example, when assessing soil quality across multiple years, each year's samples need to be comparable to enable the evaluation of potential changes or effects of management interventions.

Core Ideas

- Storage of soil samples is inevitable for large-scale soil monitoring or research projects.
- Different types of storage conditions affect measurements of soil physiochemical properties.
- We show that type of storage and duration impacts measured levels of nutrients and pH in soil samples.
- We recommend that researchers use consistent sample storage conditions for making spatial or temporal comparisons.

When performing a soil quality assessment, the cost and time required for field sampling and sample processing limit the number of soil properties that are feasible to test. Therefore, it is common that a minimum dataset (MDS) of SQIs is identified to accurately capture the overall soil quality within the given context while minimizing the workload (Bünemann et al., 2018). That is, the MDS consists of the minimum number of soil SQIs that can be used to score the quality of the soil in terms of its desired function. SQIs will vary depending on the intended land use, inherent soil properties, pre-existing soil condition, and the desired soil function(s) (Andrews et al., 2004). The Countryside Survey measures bulk density, soil carbon, pH, total nitrogen and nitrogen to carbon ratio (C:N), mineralizable nitrogen, phosphorous, a wide range of metals, and invertebrates (Emmett et al., 2010). Rutgers et al. (2009) identified nutrients as one component in their minimum dataset for measuring soil quality in the Netherlands' Biological Indicator of Soil Quality (BISQ) monitoring scheme. pH and potassium (K) are part of the indicators included in the Cornell Soil Health Assessment (Moebius-Clune et al., 2016). Emami et al. (2012) found that electrical conductivity is positively correlated with soil compaction as measured by penetration resistance.

Soil pH has been identified as a key driver of soil biodiversity (Delgado-Baquerizo & Eldridge, 2019; Ding & Eldridge, 2022), and electrical conductivity is a proxy measure of dissolved solids such as salts and exchangeable cations associated with clay fractions (Corwin & Lesch, 2005). Nitrogen is a component of amino acids and therefore necessary for all enzymatic reactions in plants, as well as contributing to photosynthesis as a major part of chlorophyll (Uchida & Silva, 2000). Soil nitrogen is a limiting component for plant growth

and ecosystem productivity (Aislabie & Deslippe, 2013) and is therefore an important indicator for agricultural soils. Potassium plays an important role in plant metabolism as it is an important part of many enzyme cofactors and is also involved in cellular transport and charge balancing (Amtmann et al., 2005). Potassium is the key determinant of cell turgor in plants, and potassium deficiency can lead to stunted root growth as this is dependent on cell turgor in the elongated cells of the root growing zone (Amtmann et al., 2005).

While the implementations of soil quality monitoring schemes, with the identification of relevant SQIs, is a valuable tool for the continued implementation of sustainable soil management, stability and continuity is required (Griffiths et al., 2018). This includes a consistent method of collecting soil samples as well as harmonized testing procedures and sample storage methods (Leeuwen et al., 2017). Schemes such as the Countryside Survey and LUCAS implement consistent sampling and laboratory methods across sites and years of study, however little focus is given to sample storage. There are many different conditions under which soil samples can be stored including refrigerated, freezer, room temperature, and drying, either at room temperature or using an oven. Different treatments and storage of soil can have different impacts on measured levels and composition of nutrients within the sample (Barbagelata & Mallarino, 2013; Ma et al., 2005; Turner & Romero, 2009). It is also possible that the amount of time between sampling and testing influences the results of nutrient tests (Hales & Ross, 2008; Turner & Romero, 2009). However, there is little recent literature available comparing different storage methods and durations (but see Forster [1995], Lundell [1987], and Kaiser et al. [2001] for older examples), and few authors report their sample storage conditions. This makes it difficult to compare results between different studies and complicates efforts to standardize methodologies.

Here, we tested the hypothesis that different storage methods (refrigerated, frozen, and oven-dried) affect the levels of nitrite (NO_2–N), nitrate (NO_3–N), ammonium (NH_4–N), and K, as well as pH of agricultural soil samples. We repeated the tests at 7 and 24 weeks to investigate potential effects of storage duration within each storage method, hypothesizing that the soil properties (NO_2, NO_3, NH_4, K, and pH) would be different at different time points.

2 | METHODS

2.1 | Sample collection

Samples were collected from a commercial strawberry (*Fragaria* × *ananassa*) plot near Faversham, Kent. Sampling was carried out in June 2022. The soil type of the area is classified as loamy (LandIS, 2024). Strawberries were growing in the ground in raised mounds, with artificial irrigation. Before strawberries, the plot had been used for blackberries (*Rubus* subg. Rubus) grown in pots. The ground was cultivated in the spring before planting and fertilizer was added through drop irrigation. Seventeen samples, each consisting of five cores from the top 15 cm of the soil, were collected from inside the strawberry mound at every third row with two samples per row. Samples were then weighed and split into three approximately equal-sized separate aliquots to be stored differently.

2.2 | Treatments and experimental design

Three storage methods were tested: refrigerated, frozen, and oven-dried. For the refrigeration treatment, samples were stored in plastic bags at 4°C, for the freezer treatment samples were frozen in plastic bags at −20°C. Frozen samples were defrosted and refrozen once during storage to allow for weighing out testing aliquots. For the oven-dried treatment, samples were placed in aluminum foil and oven-dried at 60°C until completely dry (up to 3 weeks). The dried sample aliquots were weighed before and after drying to obtain gravimetric moisture content (% moisture per gram wet soil) using Equation (1). All samples were also tested for all the chemical tests used (NO_2–N, NO_3–N, NH_4–N, K, pH) within the same week as sampling to act as a fresh control, these samples were kept refrigerated at 4°C. All samples from all three storage methods (refrigerated, oven-dried, and frozen) were then tested again at 7 and 24 weeks to reflect a medium-term and a long-term storage duration.

Equation (1) is used to calculate soil moisture expressed as percent moisture per gram of wet soil.

$$\%\ \text{Moisture} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100 \quad (1)$$

2.3 | Physiochemical testing

Samples were first weighed out into one 5-g aliquot for nitrogen compounds, one 5-g aliquot for K, and one 3-g aliquot for pH. Dried samples were weighed out dried and the previously calculated moisture content was used to adjust the subsequent nutrient concentration results to be represented as mg/kg wet weight. Each aliquot was then mixed with distilled water (1:4 w/v) and horizontally shaken at room temperature for a minimum of 2 h at 300 rpm (Stuart Orbital Incubator SI500, Cole-Parmer). Several factors influence the amounts of nitrogen extracted from soils, including extraction solution (water or salt, commonly potassium chloride or calcium chloride), concentration of extraction solution, duration of shaking, and whether samples are sieved or not (Inselbacher, 2014; Li et al.,

2012). We chose water extraction in an effort to keep the protocol simple and affordable with minimal soil disruption and sources of variation. To quantify pH of soil in an aqueous solution, a pH probe (Orion Star A211; Thermo Fisher Scientific) was calibrated with manufacturer standards of pH 4, 7, and 10. Samples were allowed to reach room temperature before measurements of pH were taken.

After shaking, the two 5-g aliquots for nutrient testing were filtered into 50-mL non-sterile centrifuge tubes using Whatman 40 filter papers in borosilicate funnels. The filtration was carried out overnight in the refrigerator (4˚C). All samples were tested for NO_2–N, NO_3–N, and NH_4–N using a discrete analyzer (Gallery, Thermo Fisher Scientific) with standard manufacturer settings and for K using a flame photometer (Jenway PFP7, Cole–Parmer). A K calibration curve was constructed with standards of known K concentrations obtained by dissolving potassium chloride (KCl) in distilled water. Only calibrations with a coefficient of determination of 0.95 or higher were accepted, and water blanks were run between each sample to ensure no cross contamination occurred.

2.4 | Statistical tests

To investigate differences between storage methods as well as between time points, a linear mixed model (LMM) was performed with R v4.3.2 (Core Team, 2023) using the lmer() function from the package *lme4* v1.1 (Bates et al., 2015). An LMM was chosen instead of analysis of variance due to some missing data points and extreme values as well as slight deviations from a normal distribution, which LMM should be more robust for. Tukey pairwise comparisons were used for post hoc testing of differences between time points within each storage method (treatment), this was done with the emmeans() function in the package *emmeans* v1.8.8 (Lenth, 2023).

3 | RESULTS

The average moisture content for the 17 samples, tested on field fresh soil prior to splitting samples into treatment aliquots, was 19.26%.

3.1 | pH

There was no significant difference in pH overall between storage methods ($p = 1$; Figure 1). There was a significant effect of time as well as an interaction between time and treatment ($p = 0.002$ and $p < 0.001$, respectively).

The most fluctuations were observed in the refrigerated treatment, where mean pH had increased significantly from 6.14 in the fresh control to 6.35 after 7 weeks ($p < 0.001$; Figure 1), and then decreased again to 5.88 at Week 24 ($p < 0.001$; Figure 1). In the dried treatment, no significant change in pH was observed between the fresh control and Week 7, but mean pH had decreased slightly at Week 24, from 6.14 in the fresh control, and 6.16 at Week 7, to 5.80 at Week 24 (Tukey pairwise comparison between fresh control and Week 24; $p = 0.02$ and between Weeks 7 and 24 $p < 0.01$; Figure 1). In the frozen treatment pH remained stable between Weeks 0 and 7, but decreased significantly (Tukey pairwise comparison $p < 0.01$) from a mean of 6.23 at Week 7 to 6.06 at Week 24 (Figure 1).

3.2 | NO_3–N

There was no significant difference between storage methods overall ($p = 1$; Figure 2). NO_3–N decreased significantly over time in all three treatments ($p < 0.001$; Figures 2 and 3), and there was a significant interaction between time and treatment ($p < 0.001$). After drying, mean NO_3–N levels significantly decreased from 31.57 mg/kg in the fresh control to 6.81 mg/kg at Week 7 ($p < 0.001$) and 2.13 mg/kg at Week 24 ($p < 0.001$). There was a further decrease in NO_3–N between Weeks 7 and 24, however this was not statistically significant ($p = 0.06$). In the fridge, NO_3–N levels had increased slightly to 39.39 mg/kg (mean) at Week 7 compared to the 31.57 mg/kg in the fresh control ($p < 0.01$) and then dropped drastically to 3.37 mg/kg (mean) at Week 24 ($p < 0.001$).

The frozen treatment was the most stable with no significant difference in NO_3–N levels between the fresh control and Week 7 ($p = 0.57$). NO_3–N then decreased from 33.94 mg/kg (mean) at Week 7 to 5.69 mg/kg at Week 24 ($p < 0.001$).

3.3 | NO_2–N

Nitrite levels were not significantly different between storage methods ($p = 1$) but there was a significant increase in NO_2–N with storage time in the dried and the frozen treatment ($p < 0.001$) and an interaction between treatment and time ($p = 0.02$).

NO_2–N levels significantly increased ($p < 0.001$) from 0.18 mg/kg (mean) in the fresh control to 0.60 mg/kg after drying (measured at Week 7). It then decreased slightly between Weeks 7 and 24 but this was not statistically significant ($p = 0.07$). There was no significant difference in NO_2–N levels between time points in the refrigerated treatment ($p > 0.05$). In the frozen treatment, NO_2–N increased slightly from 0.18 mg/kg in the fresh control to 0.47 mg/kg (mean) at Week 7 ($p = 0.04$) and then continued to increase so that the difference between the fresh control and Week 24 was highly significant (from a mean of 0.18 mg/kg to 0.84 mg/kg, $p < 0.001$), however there was no significant difference between Weeks 7 and 24 ($p = 0.33$).

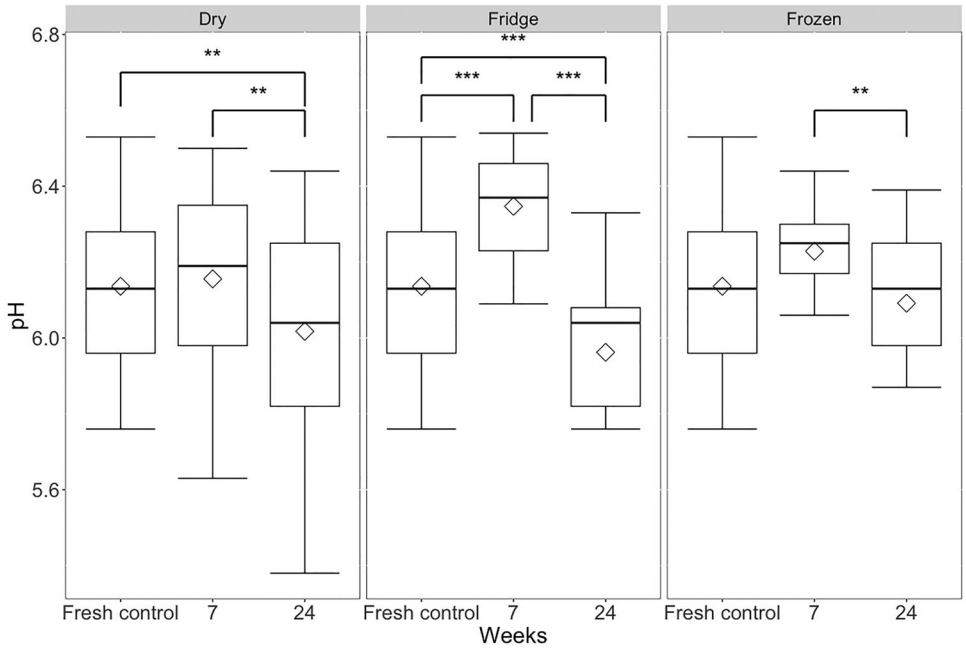

FIGURE 1 Differences in soil pH (measured in 1:4 w/v aqueous solution) in samples stored frozen (−20˚C), refrigerated (4˚C), or oven-dried (at 60˚C). Oven-dried samples were then subsequently stored at room temperature. Samples were tested three times; first as field fresh within 1 week of sampling to act as a control (fresh control) and then at 7 and 24 weeks. Brackets and stars indicate significant differences identified by Tukey pairwise comparison; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Diamond shapes indicate mean values.

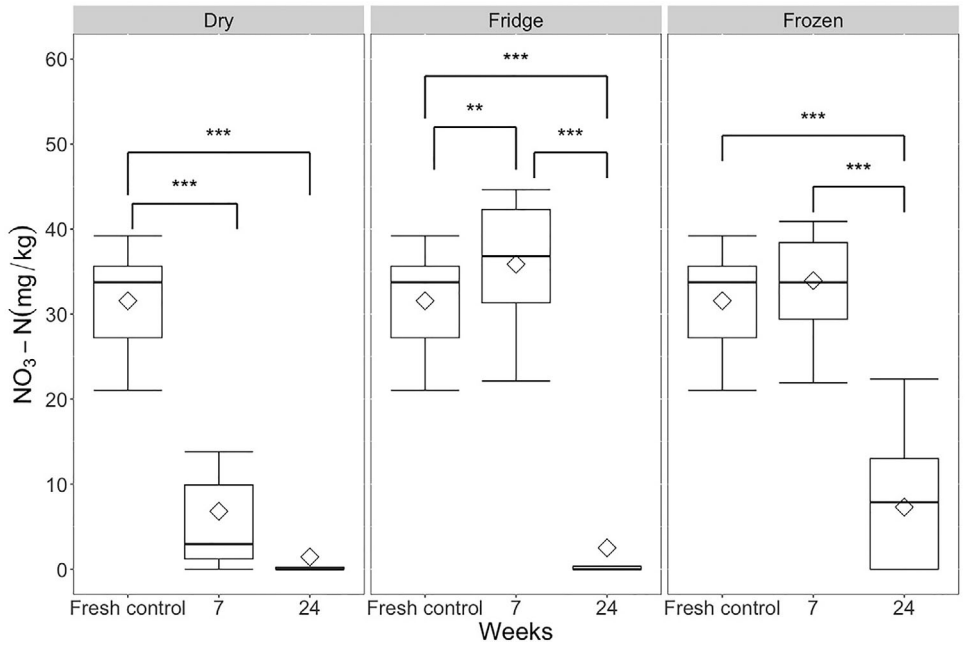

FIGURE 2 Differences in soil nitrogen as NO_3–N (measured in 1:4 w/v aqueous solution) in samples stored frozen (−20˚C), refrigerated (4˚C), or oven-dried (at 60˚C). Oven-dried samples were then subsequently stored at room temperature. Concentrations are mg/kg of field wet soil, NO_3–N concentrations measured in dried samples are reported on equivalent weight of pre-dried soil based on the gravimetric moisture content. Samples were tested three times; first as field fresh within 1 week of sampling to act as a control (fresh control) and then at 7 and 24 weeks. Brackets and stars indicate significant differences identified by Tukey pairwise comparisons; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Diamond shapes indicate mean values.

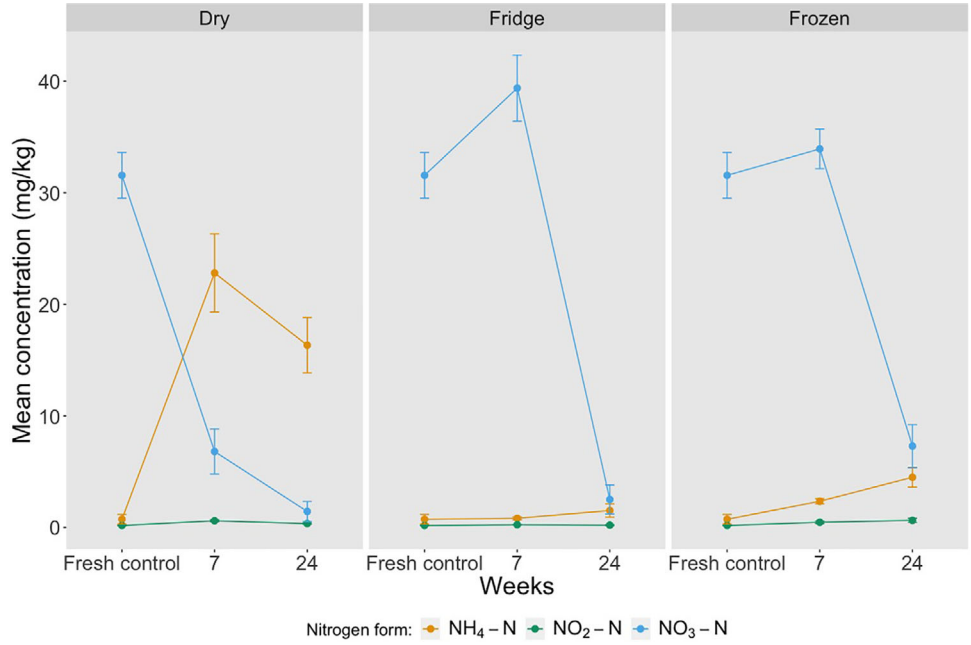

FIGURE 3 Changes in mean concentrations of soil ammonium (NH_4–N), nitrate (NO_3–N), and nitrite (NO_2–N),) in samples stored frozen (−20°C), refrigerated (4°C), or oven-dried (at 60°C). Oven-dried samples were then subsequently stored at room temperature. Concentrations are mg/kg of field wet soil, nitrogen concentrations measured in dried samples are reported on equivalent weight of pre-dried soil based on the gravimetric moisture content. Samples were tested three times; first as field fresh within 1 week of sampling to act as a control (fresh control) and then at 7 and 24 weeks. Error bars represent 1 standard error.

3.4 | NH_4–N

Overall, NH_4–N was not significantly different between storage methods ($p = 1$; Figure 4) but increased significantly over time ($p < 0.001$). There was also a significant interaction between treatment and time ($p < 0.001$).

In the dried treatment, there was a significant increase in NH_4–N from 0.73 mg/kg (mean) in the fresh control to 22.82 mg/kg at Week 7 and 15.34 mg/kg at Week 24 ($p < 0.001$), and the decrease between Weeks 7 and 24 was not significant (Figures 5 and 3). NH_4–N levels remained unchanged between fresh control and Week 7 ($p = 0.54$; Figures 5 and 3) as well as Weeks 7 and 24 ($p = 0.33$; Figures 5 and 3) in the frozen treatment but increased significantly between the 0.73 mg/kg in the fresh control and 4.56 mg/kg (mean) at Week 24 ($p = 0.04$; Figures 5 and 3). No significant differences in NH_4–N concentrations were seen in the refrigerated treatment ($p > 0.05$; Figures 5 and 3).

3.5 | Potassium

There was no overall effect of storage method ($p = 1$) on K levels but there was a significant effect of time overall and a significant interaction between treatment and time ($p < 0.001$ for both).

K was the most stable in the frozen treatment with no significant difference between the fresh control and Week 7 ($p = 1$; Figure 6). There was, however, a significant increase in K from 60.27 mg/kg (mean) at Week 7 to 70.99 mg/kg (mean) at Week 24 ($p = 0.03$). In the refrigerator, K levels increased significantly from 60.49 mg/kg (mean) in the fresh control to 76.53 mg/kg (mean) at Week 7 ($p < 0.001$) and 76.98 at Week 24 (pairwise comparison of fresh control and Week 24, $p < 0.01$; Figure 6). In the oven-dried treatment, K levels decreased significantly from the 60.49 mg/kg (mean) in the fresh control to 50.12 mg/kg at Week 7 ($p > 0.01$; Figure 6) and then increased significantly to 69.49 mg/kg (mean) at Week 24 (pairwise comparison between Weeks 7 and 24, $p < 0.001$; Figure 6). K levels in the dry treatment were also significantly higher ($p < 0.01$) at Week 24 (69.49 mg/kg) compared to the fresh control (60.49 mg/kg).

4 | DISCUSSION

We investigated the effect of sample storage method on measured levels of soil pH, electrical conductivity, nitrogen (NO_3–N, NO_2–N, NH_4–N), and potassium at three time points over a 24-week period (0 [control], 7, and 24 weeks). The results demonstrate clear differences between the storage methods (oven-dried, refrigerated, and frozen) as well as variations in measurements between time points. Other studies

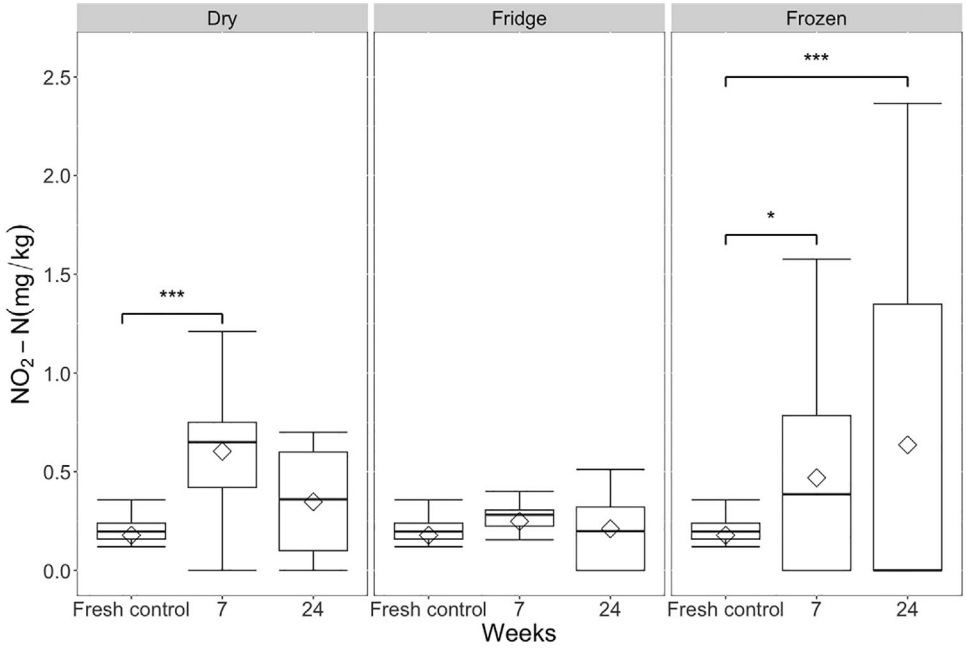

FIGURE 4 Differences in soil nitrogen as NO_2–N (measured in 1:4 w/v aqueous solution) in samples stored frozen (−20˚C), refrigerated (4˚C), or oven-dried (at 60˚C). Oven-dried samples were then subsequently stored at room temperature. Concentrations are mg/kg of field wet soil, NO_2–N concentrations measured in dried samples are reported on equivalent weight of pre-dried soil based on the gravimetric moisture content. Samples were tested three times; first as field fresh within 1 week of sampling to act as a control (fresh control) and then at 7 and 24 weeks. Brackets and stars indicate significant differences identified by Tukey pairwise comparisons; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Diamond shapes indicate mean values.

FIGURE 5 Differences in soil nitrogen as NH_4–N (measured in 1:4 w/v aqueous solution) in samples stored frozen (−20˚C), refrigerated (4˚C), or oven-dried (at 60˚C). Oven-dried samples were then subsequently stored at room temperature. Concentrations are mg/kg of field wet soil, NH_4–N concentrations measured in dried samples are reported on equivalent weight of pre-dried soil based on the gravimetric moisture content. Samples were tested three times; first as field fresh within 1 week of sampling to act as a control (fresh control) and then at 7 and 24 weeks. Brackets and stars indicate significant differences identified by Tukey pairwise comparisons; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Diamond shapes indicate mean values.

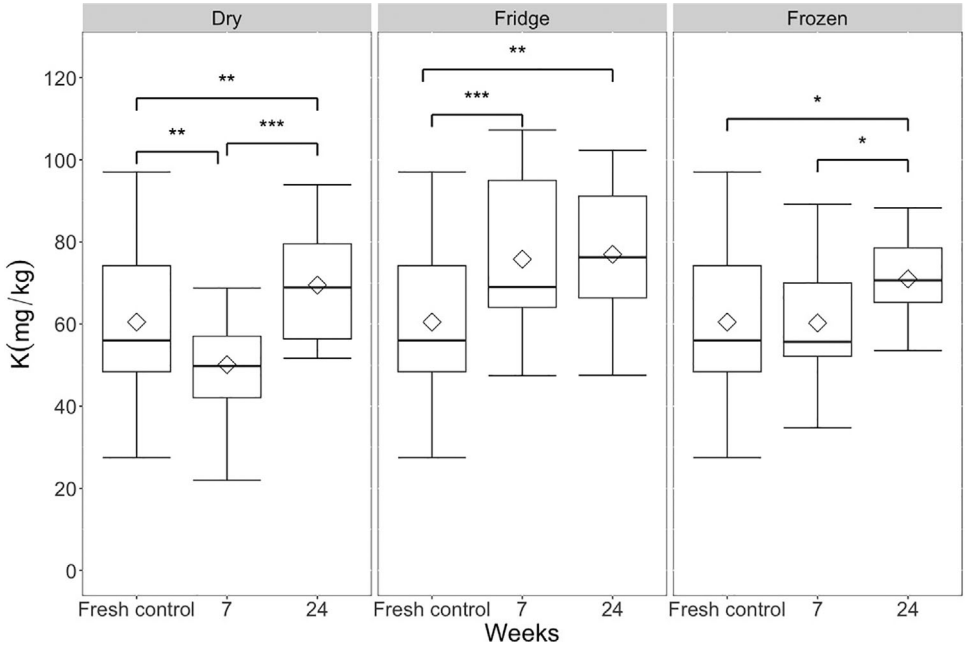

FIGURE 6 Differences in soil nitrogen as potassium (K) (measured in 1:4 w/v aqueous solution) in samples stored frozen (−20˚C), refrigerated (4˚C), or oven-dried (at 60˚C). Oven-dried samples were then subsequently stored at room temperature. Concentrations are mg/kg of field wet soil, K concentrations measured in dried samples are reported on equivalent weight of pre-dried soil based on the gravimetric moisture content. Samples were tested three times; first as field fresh within 1 week of sampling to act as a control (fresh control) and then at 7 and 24 weeks. Brackets and stars indicate significant differences identified by Tukey pairwise comparisons; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Diamond shapes indicate mean values.

have also found that storage method, as well as other experimental conditions, affect physiochemical measurements of soil samples (Kaiser et al., 2001).

4.1 | pH

In the refrigerated treatment, pH had increased at Week 7 and then decreased at Week 24 to similar levels as the fresh control. The same pattern can be seen in the freezer but the difference between the fresh control and Week 7 was smaller and nonsignificant. Additionally, the decrease between Weeks 7 and 24 in the frozen treatment was smaller than in the refrigerator, and there was no significant difference between the fresh control and Week 24 in the freezer. pH was more stable in the dried treatment compared to the refrigerated as no significant difference was observed between the fresh control and Week 7. There was, however, a small decrease at Week 24 compared to the fresh control and Week 7. Since pH fluctuated the least in the freezer, followed by the dried treatment, those are suggested as the preferred storage method when measuring pH, rather than refrigeration. However, as we observed no significant difference overall between storage methods for pH and the differences that we did observe, while statistically significant, were very small (maximum difference of 0.34 points in the refrigerated treatment). Since most crops grow well when soil pH is approximately neutral (Carter & Gregorich, 2007), these small changes in pH should not reflect any major changes in growing conditions. On the other hand, since pH is measured on a logarithmic scale, even small changes in measured pH can reflect relevant changes in soil acidity; we therefore suggest storing soil samples in the freezer or oven-dried when striving to obtain agriculturally or ecologically relevant pH measurements.

Kissel et al. (2009) showed that soil moisture content affects pH measurements due to its effect on the salt concentration of the soil solution. One possible explanation for the results presented herein is that drying the soil samples result in a more stable salinity compared to refrigeration, due to lack of variations in the soil solution due to varying water contents, which in turn leads to a more stable pH. Additionally, since samples were diluted in a 1:4 soil:water ratio, dried samples should have a more accurate water content during testing, since they can be expected to have less variation in starting moisture content. Similarly, frozen samples might undergo fewer changes in soil moisture since any water in the soil will be stabilized as ice and therefore not evaporate.

Another important point is that there are several other factors that can influence measurements of pH, such as soil:water ratio, whether soil is diluted in water or another solution such as CaCl, and temperature (Fabian et al., 2014; Kissel et al., 2009; Miller & Kissel, 2010; Thunjai et al., 2001). Our

results simply add sample storage as one of the many factors that should be considered when planning sample handling for standardizations of studies.

4.2 | Nitrogen

Nitrate was very unstable in all three storage methods with the largest fluctuations in the dry treatment (Figures 2 and 3). It remained most stable in the freezer, with no significant difference in NO_3 levels between the fresh control and Week 7 ($p = 0.57$; Figure 2). These results suggest that agricultural soil samples should be stored in the freezer, and not for longer than 7 weeks, to ensure accurate measurements of NO_3–N levels. Since the change between the fresh control and Week 7 was relatively small in the refrigerator (7.82 mg/kg difference; Figure 2), this storage method should also be acceptable for short-term storage when testing NO_3–N levels. Further studies should investigate the differences in NO_3 levels at more frequent intervals between 7 and 24 weeks to determine the maximum storage time.

As denitrification (the reduction of nitrate to gaseous N_2) is known to increase with temperature (Dai et al., 2020), this is one possible mechanism by which the nitrate is being lost when samples are dried. Microbial activity also affects denitrification rates and microbial activity is in turn affected by temperature (Černohlávková et al., 2009), this could help explain why freezing results in slightly less NO_3 lost, as freezing would likely decrease microbial activity as well as reduce overall denitrification (Tzanakakis et al., 2020). However, Černohlávková et al. (2009) found highly variable effects of storage conditions on microbial activity and nitrogen fluxes, meaning we cannot, with the data available in this study, know with certainty whether microbial activity explains our results.

Nitrite remained largely unchanged in the refrigerator but increased significantly after drying (0.18 mg/kg–0.60 mg/kg; $p < 0.001$), however it remained stable once dried. Freezing resulted in a continuous increase of NO_2–N such that the levels at Week 24 were 0.66 mg/kg higher compared to the fresh control. While these changes were significant, the total NO_2–N was very low and since NO_2–N is not as readily acquired by plants as NO_3–N and NH_4–N (Zayed et al., 2023), it is unlikely that a change of <1 mg/kg reflects an agriculturally important difference. Nonetheless, for studies where accurate measurements of soil NO_2–N are required, we suggest refrigeration as the optimal soil sample storage method.

Ammonium increased slightly over time in the freezer but remained stable in the refrigerated conditions. After drying, NH_4 increased significantly but remained largely stable once dried. Since NH_4–N increased and NO_3–N decreased in all three treatments (Figure 3), it is possible that some NO_3–N was converted to NH_4–N. It is not possible, however, to confirm the exact mechanisms by which the different nitrogen compounds are being lost or gained with the data presented herein and neither was that the intended scope of the study.

The results presented here suggest that there is a risk of overestimating NH_4–N levels in dried soil samples, and we suggest refrigeration of soil samples intended for NH_4–N testing. It would be of interest to further investigate changes in NH_4–N levels over a longer storage duration than the 24 weeks tested here.

In summary, optimal soil storage method for nitrogen testing depends on which forms of nitrogen are being targeted as well as how long the samples need to be stored for. For NO_3–N, freezing would be the preferred method, however for NH_4–N and NO_2–N, refrigeration is optimal. If testing for all, or two of, the three compounds tested herein, samples could be split and stored in a freezer for NO_3–N testing and in the fridge for NH_4–N and NO_2–N testing. Alternatively, if samples are going to be tested within 7 weeks of sampling, refrigeration should yield acceptable results for NO_3–N and whole samples could therefore be stored in a fridge, as long as researchers are aware that NO_3–N might be slightly overestimated. These suggestions are based on agricultural soil and we suggest caution when applying them to soils from different land uses as well as different environments. Additionally, Inselsbacher et al. (2011) showed that different amounts of N compounds were recovered by different testing methods and further work comparing the combined effect of storage and soil test method would be of interest.

4.3 | Potassium

Potassium levels remained stable for the first 7 weeks in the frozen treatment but increased significantly between Weeks 7 and 24. In the refrigerated treatment, the opposite was observed; K increased by 16.04 mg/kg between the fresh control and Week 7, which was statistically significant ($p < 0.001$). Once chilled, however, there was no significant difference between Weeks 7 and 24. Potassium levels decreased significantly ($p < 0.001$) by approximately 10 mg/kg between the fresh control and Week 7 in the dry treatment, and then increased by approximately 20 mg/kg from Week 7 to 24. This means that the difference in K concentration between the fresh control and Week 24 in the dry treatment was only 10 mg/kg which suggests that for long-term storage, drying can be a feasible option. However, it is not clear whether K concentration in soil samples would continue to fluctuate if samples are stored dried for longer than 24 weeks. Some authors have reported that if the initial levels of exchangeable K in a soil are high, the soil tends to fix K in mineral form that are non-extractable, whereas soils with a low amount of initial exchangeable K tend to release K when drying (Zörb et al., 2014). Therefore, it is possible that different samples will respond differently to drying,

however, other studies have reached the same results as the ones presented here (Barbagelata & Mallarino, 2013; Gupta & Rorison, 1974). Further studies are needed to compare the behavior of K in different storage conditions for different types of soils before any general recommendations and guidelines can be made.

These results suggest that the best storage method for agricultural samples for K testing is freezing as K fluctuations were smallest in this storage condition.

Other factors that influence levels of extractable K in soil samples include organic matter, and the sum of Ca plus Mg (Barbagelata & Mallarino, 2013), which were not measured in this study. We conclude that measurements of soil K are subject to a complex set of interacting variables, but that standardization of sample storage can remove one source of variation.

5 | CONCLUSIONS

Soil monitoring schemes are vital for the development of sustainable land-use practices and associated policies. However, for accurate comparisons between years or seasons, both field and laboratory methodologies should be standardized to eliminate variations in test results due to differences in sampling or analytical techniques. While existing schemes such as the UK Countryside Survey and the EU Land Use Cover Area Survey implement standardized sampling and analytical methods, storage of soil samples is rarely considered in the literature.

The results presented here indicate that storage method as well as duration affect the measurements of physiochemical properties in soil samples. When comparing sampling sites or plots from the same site, it is paramount that samples are not only collected in a standardized and repeatable way, but that sample storage is approached with the same rigor. We recommend freezing when testing soil samples for NO_3–N and K, refrigeration when testing NH_4–N, and NO_2–N, and oven drying or freezing when testing pH. For NO_3–N, minimizing storage time (even when freezing samples) is recommended, as NO_3–N levels had significantly decreased after 7 weeks.

AUTHOR CONTRIBUTIONS

Maya Sollen-Norrlin: Conceptualization; data curation; formal analysis; methodology; project administration; visualization; writing—original draft; writing—review and editing. **Naomi Laura Jane Rintoul-Hynes**: Conceptualization; methodology; project administration; supervision; writing—review and editing.

ACKNOWLEDGMENTS

The authors would like to extend their gratitude to Dr Catherine Sanders for advice on statistics and feedback on the R code. The authors would further like to thank Canterbury Christ Church University for funding this study through the Graduate Scholarship. The authors would also like to give heartfelt thanks to Edward Vinson Ltd. for allowing us access to their site to collect soil samples.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available on zenodo.org (https://doi.org/10.5281/zenodo.10626039).

ORCID

Maya Sollen-Norrlin https://orcid.org/0009-0005-9662-0283

REFERENCES

Aislabie, J., & Deslippe, J. R. (2013). Soil microbes and their contribution to soil services. In J. R. Dymond (Ed.), *Ecosystem services in New Zealand: Conditions and trends* (Vol. 12, pp. 143–161). Manaaki Whenua Press.

Amtmann, A., Hammond, J. P., Armengaud, P., & White, P. J. (2005). Nutrient sensing and signalling in plants: Potassium and phosphorus. *Advances in Botanical Research*, *43*, 209–257. https://doi.org/10.1016/S0065-2296(05)43005-0

Andrews, S. S., Karlen, D. L., & Cambardella, C. A. (2004). The soil management assessment framework. *Soil Science Society of America Journal*, *68*(6), 1945–1962. https://doi.org/10.2136/sssaj2004.1945

Bailey, T., Robinson, N., Farrell, M., Macdonald, B., Weaver, T., Antille, D. L., Chin, A., Brackin, R., & Abiven, S. (2021). Storage of soil samples leads to over-representation of the contribution of nitrate to plant-available nitrogen. *Soil Research*, *60*(1), 22–32. https://doi.org/10.1071/SR21013

Barbagelata, P. A., & Mallarino, A. P. (2013). Field correlation of potassium soil test methods based on dried and field-moist soil samples for corn and soybean. *Soil Science Society of America Journal*, *77*(1), 318–327. https://doi.org/10.2136/sssaj2012.0253

Bates, D., Mächler, M., Bolker, B., & Walker, S. R. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Brevik, E. C., Pereg, L., Steffan, J. J., & Burgess, L. C. (2018). Soil ecosystem services and human health. *Current Opinion in Environmental Science & Health*, *5*, 87–92. https://doi.org/10.1016/j.coesh.2018.07.003

Bünemann, E. K., Bongiorno, G., Bai, Z., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T. W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J. W., & Brussaard, L. (2018). Soil quality—A critical review. *Soil Biology and Biochemistry*, *120*, 105–125. https://doi.org/10.1016/j.soilbio.2018.01.030

Carter, M. R., & Gregorich, E. G. (Eds.). (2007). *Soil sampling and methods of analysis*. CRC Press.

Černohlávková, J., Jarkovský, J., Nešporová, M., & Hofman, J. (2009). Variability of soil microbial properties: Effects of sampling, handling and storage. *Ecotoxicology and Environmental Safety*, *72*(8), 2102–2108. https://doi.org/10.1016/j.ecoenv.2009.04.023

Core Team R. (2023). R: A language and environment for statistical computing [Computer software]. R Foundation for Statistical Computing. https://www.r-project.org/

Corwin, D. L., & Lesch, S. M. (2005). Apparent soil electrical conductivity measurements in agriculture. *Computers and Electronics in Agriculture*, *46*(1), 11–43. https://doi.org/10.1016/j.compag.2004.10.005

Dai, Z., Yu, M., Chen, H., Zhao, H., Huang, Y., Su, W., Xia, F., Chang, S. X., Brookes, P. C., Dahlgren, R. A., & Xu, J. (2020). Elevated temperature shifts soil N cycling from microbial immobilization to enhanced mineralization, nitrification and denitrification across global terrestrial ecosystems. *Global Change Biology*, *26*(9), 5267–5276. https://doi.org/10.1111/gcb.15211

Delgado-Baquerizo, M., & Eldridge, D. J. (2019). Cross-biome drivers of soil bacterial alpha diversity on a worldwide scale. *Ecosystems*, *22*(6), 1220–1231. https://doi.org/10.1007/s10021-018-0333-2

Delgado-Baquerizo, M., Reich, P. B., Trivedi, C., Eldridge, D. J., Abades, S., Alfaro, F. D., Bastida, F., Berhe, A. A., Cutler, N. A., Gallardo, A., García-Velázquez, L., Hart, S. C., Hayes, P. E., He, J. Z., Hseu, Z. Y., Hu, H. W., Kirchmair, M., Neuhauser, S., Pérez, C. A., … Singh, B. K. (2020). Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution*, *4*(2), 210–220. https://doi.org/10.1038/s41559-019-1084-y

Department for Food, Environment, and Rural Affairs (DEFRA), & Rt Hon Gove, M. (2018). *A green future: Our 25 year plan to imptove the environment*. Government of the United Kingdom. https://www.gov.uk/government/publications/25-year-environment-plan

Ding, J., & Eldridge, D. J. (2022). Drivers of soil biodiversity vary with organism type along an extensive aridity gradient. *Applied Soil Ecology*, *170*, 104271. https://doi.org/10.1016/j.apsoil.2021.104271

Emami, H., Neyshabouri, M. R., & Shorafa, M. (2012). Relationships between some soil quality indicators in different agricultural soils from Varamin, Iran. *Journal of Agricultural Science and Technology*, *14*(4), 951–959.

Emmett, B. A., Reynolds, B., Chamberlain, P. M., Rowe, E., Spurgeon, D., Brittain, S. A., Frogbrook, Z., Hughes, S., Lawlor, A. J., Poskitt, J., Potter, E., Robinson, D. A., Scott, A., Wood, C., & Woods, C. (2010). Countryside survey: Soils report from 2007 (CS Technical Report No. 9/07, CEH Project No. C03259). UK Centre for Ecology & Hydrology.

Fabian, C., Reimann, C., Fabian, K., Birke, M., Baritz, R., & Haslinger, E. (2014). GEMAS: Spatial distribution of the pH of European agricultural and grazing land soil. *Applied Geochemistry*, *48*, 207–216. https://doi.org/10.1016/j.apgeochem.2014.07.017

FAO, ITPS, GSBI, SCBD, & EC. (2020). *State of knowledge of soil biodiversity—Status, challenges and potentialities*. FAO. https://doi.org/10.4060/cb1928en

Forster, J. C. (1995). Soil sampling, handling, storage and analysis. In K. Alef, & P. Nannipieri (Eds.), *Methods in applied soil microbiology and biochemistry* (pp. 49–121). Academic Press. https://doi.org/10.1016/B978-012513840-6/50018-5

Griffiths, B. S., Faber, J., & Bloem, J. (2018). Applying soil health indicators to encourage sustainable soil use: The transition from scientific study to practical application. *Sustainability*, *10*(9), 3021. https://doi.org/10.3390/su10093021

Gupta, P. L., & Rorison, I. H. (1974). Effects of storage on the soluble phosphorus and potassium content of some Derbyshire soils. *Journal of Applied Ecology*, *11*(3), 1185–1192. https://doi.org/10.2307/2401772

Hales, H. C., & Ross, D. S. (2008). Drastic short-term changes in the isotopic composition of soil nitrate in forest soil samples. *Soil Science Society of America Journal*, *72*(6), 1645–1652. https://doi.org/10.2136/sssaj2006.0293

Haney, R. L., Haney, E. B., Smith, D. R., Harmel, R. D., & White, M. J. (2018). The soil health tool—Theory and initial broad-scale application. *Applied Soil Ecology*, *125*, 162–168. https://doi.org/10.1016/j.apsoil.2017.07.035

Huang, S., Wang, P., Yamaji, N., & Ma, J. F. (2020). Plant nutrition for human nutrition: Hints from rice research and future perspectives. *Molecular Plant*, *13*(6), 825–835. https://doi.org/10.1016/j.molp.2020.05.007

Inselsbacher, E. (2014). Recovery of individual soil nitrogen forms after sieving and extraction. *Soil Biology and Biochemistry*, *71*, 76–86. https://doi.org/10.1080/00103624.2012.639108

Inselsbacher, E., Öhlund, J., Jämtgård, S., Huss-Danell, K., & Näsholm, T. (2011). The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. *Soil Biology and Biochemistry*, *43*(6), 1321–1332. https://doi.org/10.1016/j.soilbio.2011.03.003

Jones, A., Montanarella, L., & Tóth, G. (2013). *LUCAS topsoil survey—Methodology, data and results*. European Union Publications. https://data.europa.eu/doi/10.2788/97922

Kaiser, K., Kaupenjohann, M., & Zech, W. (2001). Sorption of dissolved organic carbon in soils: Effects of soil sample storage, soil-to-solution ratio, and temperature. *Geoderma*, *99*(3), 317–328. https://doi.org/10.1016/S0016-7061(00)00077-X

Kassambara, A. (2023). rstatix: Pipe-friendly framework for basic statistical tests (Version 0.7.2) [Computer software]. https://CRAN.R-project.org/package-rstatix

Kissel, D. E., Sonon, L., Vendrell, P. F., & Isaac, R. A. (2009). Salt concentration and measurement of soil pH. *Communications in Soil Science and Plant Analysis*, *40*(1–6), 179–187. https://doi.org/10.1080/00103620802625377

Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., & Lombi, E. (2019). Soil and the intensification of agriculture for global food security. *Environment International*, *132*, 105078. https://doi.org/10.1016/j.envint.2019.105078

LandIS. (2024). *Soilscapes viewer*. Cranfield University. https://www.landis.org.uk/soilscapes/

Leeuwen, J. P. V., Saby, N. P. A., Jones, A., Louwagie, G., Micheli, E., Rutgers, M., Schulte, R. P. O., Spiegel, H., Toth, G., & Creamer, R. E. (2017). Gap assessment in current soil monitoring networks across ˚Europe for measuring soil functions. *Environmental Research Letters*, *12*(12), 124007. https://doi.org/10.1088/1748-9326/aa9c5c

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. *Nature Reviews Earth & Environment*, *1*(10), 544–553. https://doi.org/10.1038/s43017-020-0080-8

Lenth, R. (2023). emmeans: Estimated marginal means, aka least-squares means. R package version 1.8.8. [Computer software]. https://CRAN.R-project.org/package=emmeans

Li, K. Y., Zhao, Y. Y., Yuan, X. L., Zhao, H. B., Wang, Z. H., Li, S. X., & Malhi, S. S. (2012). Comparison of factors affecting soil nitrate nitrogen and ammonium nitrogen extraction. *Communications in Soil Science and Plant Analysis*, *43*(3), 571–588. https://doi.org/10.1080/00103624.2012.639108

Lundell, Y. (1987). Nutrient variation in forest soil samples due to time of sampling and method of storage. *Plant and Soil*, *98*(3), 363–375. https://doi.org/10.1007/BF02378358

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. https://doi.org/10.1081/PLN-200055536

Miller, R. O., & Kissel, D. E. (2010). Comparison of soil pH methods on soils of North America. *Soil Science Society of America Journal*, *74*(1), 310–316. https://doi.org/10.2136/sssaj2008.0047

Moebius-Clune, B. N., Moebius-Clune, D. J., Gugino, B. K., Idowu, O. J., Schindelbeck, R. R., Ristow, A. J., van Es, H. M., Thies, J. E., & Shayler, H. A. (2016). *Comprehensive assessment of soil health—The Cornell framework* (ed. 3.2). Cornell University.

Oliver, D. P., Bramley, R. G. V., Riches, D., Porter, I., & Edwards, J. (2013). Review: Soil physical and chemical properties as indicators of soil quality in Australian viticulture: Indicators for soil quality. *Australian Journal of Grape and Wine Research*, *19*(2), 129–139. https://doi.org/10.1111/ajgw.12016

Pereira, P., Bogunovic, I., Muñoz-Rojas, M., & Brevik, E. C. (2018). Soil ecosystem services, sustainability, valuation and management. *Current Opinion in Environmental Science & Health*, *5*, 7–13. https://doi.org/10.1016/J.COESH.2017.12.003

Rutgers, M., Schouten, A. J., Bloem, J., Van Eekeren, N., De Goede, R. G. M., Jagers Op Akkerhuis, G. A. J. M., Van Der Wal, A., Mulder, C., Brussaard, L., & Breure, A. M. (2009). Biological measurements in a nationwide soil monitoring network. *European Journal of Soil Science*, *60*(5), 820–832. https://doi.org/10.1111/j.1365-2389.2009.01163.x

Sims, J. T., Cunningham, S. D., & Sumner, M. E. (1997). Assessing soil quality for environmental purposes: Roles and challenges for soil scientists. *Journal of Environmental Quality*, *26*(1), 20–25. https://doi.org/10.2134/jeq1997.00472425002600010004x

Stavi, I., & Lal, R. (2015). Achieving zero net land degradation: Challenges and opportunities. *Journal of Arid Environments*, *112*(Part A), 44–51. https://doi.org/10.1016/J.JARIDENV.2014.01.016

Thunjai, T., Boyd, C. E., & Dube, K. (2001). Poind soil pH measurement. *Journal of the World Aquaculture Society*, *32*(2), 141–152. https://doi.org/10.1111/j.1749-7345.2001.tb01089.x

Turner, B. L., & Romero, T. E. (2009). Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal*, *73*(6), 1972–1979. https://doi.org/10.2136/sssaj2008.0407

Tzanakakis, V. A., Taylor, A. E., & Bottomley, P. J. (2020). Impact of freeze-thaw on the contributions of AOA and AOB to N-flush induced nitrification in meadow soils. *Soil Biology and Biochemistry*, *150*, 108015. https://doi.org/10.1016/j.soilbio.2020.108015

Uchida, R., & Silva, J. A. (Eds.) (2000). Essential nutrients for plant growth: Nutrient functions and deficiency symptoms. In *Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture* (pp. 31–55). College of Tropical Agriculture and Human Resources.

Zayed, O., Hewedy, O. A., Abdelmoteleb, A., Ali, M., Youssef, M. S., Roumia, A. F., Seymour, D., & Yuan, Z. C. (2023). Nitrogen journey in plants: From uptake to metabolism, stress response, and microbe interaction. *Biomolecules*, *13*(10), 1443. https://doi.org/10.3390/biom13101443

Zörb, C., Senbayram, M., & Peiter, E. (2014). Potassium in agriculture—Status and perspectives. *Journal of Plant Physiology*, *171*(9), 656–669. https://doi.org/10.1016/j.jplph.2013.08.008

How to cite this article: Sollen-Norrlin, M., & Rintoul-Hynes, N. L. J. (2024). Soil sample storage conditions affect measurements of pH, potassium, and nitrogen. *Soil Science Society of America Journal*, 1–12. https://doi.org/10.1002/saj2.20653