Short-Term Changes in Extractable Inorganic Nutrients during Storage of Tropical Rain Forest Soils

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The nutrient status of tropical forests is commonly assessed by measuring inorganic nutrients extracted from soil, yet samples from remote research sites may be stored for prolonged periods of time before analysis. We assessed the influence of soil storage conditions on extractable nutrients in three lowland tropical forests soils from the Republic of Panama. The soils spanned a strong rainfall gradient and contained contrasting chemical and physical properties. Storage treatments were: (i) room temperature (22°C in the dark), (ii) refrigerated (4°C in the dark), (iii) air dried (10 d at 22°C and 55% humidity), and (iv) frozen (−35°C). Ammonium and NO_3 were extremely unstable and concentrations changed considerably within hours of sampling. Phosphate extracted by anion-exchange membranes also changed rapidly following sampling, although cations (Ca, K, and Mg) extracted in Mehlich-3 solution were less influenced by storage. Soil pH declined slowly in all samples during field-moist storage (4 and 22°C). Freezing and air drying generally caused significant changes in extractable nutrients, although the effects varied among soils and nutrients. We therefore conclude that inorganic nutrients should be extracted from tropical forest soils within 24 h of sampling, and preferably on the day of sampling for N fractions, to ensure that values represent field conditions. Where this is not possible, rapid air drying or storage of field-moist samples may be acceptable for some measurements (e.g., PO_4, cations, pH), but are unlikely to provide realistic measurements of inorganic N.

Abbreviations: AEM, anion exchange membrane; ICP–OES, inductively coupled plasma optical-emission spectrometry.

Soils are often stored before analysis. This can involve air drying, freezing, or refrigeration, while samples can also be effectively "stored" at ambient temperature for hours to days during transport from the field site to the laboratory (or to suitable refrigeration or drying facilities). This can influence subsequent concentrations of extractable inorganic nutrients, including NH_4 and NO_3 (Birch, 1960; Mulvaney, 1996), PO_4 (Sparling et al., 1985; Turner and Haygarth, 2003), K (Gupta and Rorison, 1974; Luo and Jackson, 1985), Mn (Boken, 1952; Nelson, 1977), and SO_4 (Comfort et al., 1991).

Changes in inorganic N during storage at field moisture have been well known for many years (e.g., Munro and Mackay, 1964) and are used to determine potentially mineralizable N (Hart et al., 1994). This has implications for studies involving the isotopic composition of soil NO_3, which can change considerably within hours of sampling (Hales and Ross, 2008). Phosphorus is also sensitive to storage, and marked changes can occur following refrigeration (Chapman et al., 1997), freezing

(Ron Vaz et al., 1994), and air drying (Turner and Haygarth, 2001; 2003). The latter is commonly assumed to stabilize samples, although changes in both N and P fractions can continue during long-term storage of air-dried soils (e.g., Nevo and Hagin, 1966; Turner, 2005).

Changes in extractable nutrients during storage pose a particular challenge in the study of tropical forest soils. Research sites are often remote from laboratory facilities, so soil samples may require long periods of transport and storage before preparation and analysis. The effects of storage have rarely been assessed for tropical soils (although see Birch, 1960), yet results from experiments on temperate soils, which often undergo large seasonal fluctuations in temperature, may not readily translate to lowland tropical forest soils, which typically experience a stable, warm temperature without prolonged periods of cold or freezing (although they may experience a strong dry period). Tropical soils may therefore be influenced by cold or frozen storage to a greater extent than temperate or arctic soils.

We assessed the influence of short-term storage on extractable inorganic nutrients in three soils under lowland tropical rain forest in Panama. The soils spanned a strong rainfall gradient and contained contrasting concentrations of extractable nutrients. Our aim was to determine the most acceptable conditions for storage before the extraction of tropical forest soils to approximate nutrient concentrations in the field.

MATERIALS AND METHODS

Site Description

Three soils were sampled from central Panama (Table 1). The soils spanned a strong rainfall gradient and contained contrasting physical

and chemical properties, including pH (3.63–5.89 in de-ionized water), organic matter (26.2–50.1 g C kg^{-1}), and total P (0.297–0.598 g P kg^{-1}). All three sites were under lowland rain forest and were adjacent to a 1-ha forest census plot. Detailed information on the forest communities in two of these plots was reported previously (Fort Sherman, plot code P02; Pipeline Road, plot code P15; Pyke et al., 2001).

The air temperature in the region is stable throughout the year. The mean annual temperature on Barro Colorado Island, a few kilometers from Pipeline Road, is 26°C and varies by only 1°C throughout the year (Windsor, 1990). Mean annual rainfall varies from 1811 mm at Albrook to 3072 mm at Fort Sherman (Table 1). There is a strong dry season from January to April, although the duration varies among the three sites. The mean dry-season length, calculated from a network of rainfall stations throughout the area, is 148 d at Albrook, 133 d at Pipeline Road, and 118 d at Fort Sherman (Engelbrecht et al., 2007).

Soil Sampling and Preparation

Soil samples were taken during the wet season. The Albrook soil was sampled on 5 June 2006, while the Pipeline Road and Fort Sherman soils were sampled on 22 Aug. 2007. At each location, approximately 10 kg of soil was taken to 10-cm depth from an area adjacent to the forest census plot and returned immediately to the laboratory (see below). This was within 30 min for the Albrook soil, which was then prepared and analyzed on the day of collection. Transportation time was longer for the Pipeline Road (1 h) and Fort Sherman (4 h) soils, so samples were prepared and separated into storage treatments (see below) on the day of collection, but the first analyses were conducted the following morning (within 19 and 22 h of collection for the Pipeline Road and Fort Sherman soils, respectively).

Upon return to the laboratory, the soils were screened (<9-mm mesh) to break up large aggregates, and visible stones and roots were removed by hand. The Albrook soil in particular contained many fine roots and small stones. The samples were not further sieved due to the difficulty in sieving field-moist soils that are rich in clay. Each soil was then split into four treatments designed to simulate commonly used storage protocols: (i) storage in the dark at ambient laboratory temperature and humidity (22 ± 0.5°C and 55 ± 5%, respectively); (ii) storage in the dark at 4°C; (iii) storage frozen at −35°C; and (iv) air drying at ambient laboratory temperature and humidity for 10 d.

Each treatment was replicated four times for each soil. Samples were stored in sealed plastic bags, with the air-dried samples stored at ambient laboratory temperature. Frozen and air-dried samples contained approximately 200 g of soil per replicate and were analyzed 4 wk after sampling. Samples stored at 4 and 22°C contained approximately 800 g of soil per replicate and were subsampled periodically for analysis. The Albrook soil was analyzed on the day of sampling, then again 1, 3, 7, 15, 35, and 95 d after sampling. The Sherman and Pipeline soils were analyzed on the day following sampling, then again 3, 7, 15, and 28 d after sampling. Frozen soils were thawed overnight at 4°C before analysis the following morning.

Soil Analysis to Determine Storage Effects

Soil moisture was determined by drying a 20-g sample for 24 h at 105°C. Values at the time of sampling were: Albrook, 31%; Pipeline

Table 1. Site characteristics and properties of three soils under lowland tropical rain forest in central Panama. Site information for Pipeline Road and Fort Sherman is from Pyke et al. (2001) and rainfall data are from Engelbrecht et al. (2007).

Parameter	Albrook	Pipeline Road	Fort Sherman
Latitude	8°58′37″ N	9°9′42″ N	9°19′23″ N
Longitude	79°33′50″ W	79°44′43″ W	79°57′43″ W
Geology	La Boca formation (marine sedimentary)	Gatuncillo formation (marine sedimentary)	Chagres sandstone
Elevation, m	53	70	84
Rainfall, mm yr^{-1}	1811	2455	3072
Soil pH (H$_2$O)	3.63	5.89	5.25
Topsoil texture	clay	clay loam	clay
Sand, %	30	36	15
Silt, %	16	31	24
Clay, %	54	33	62
Total C, g kg^{-1}	50.1	26.2	30.6
Total N, g kg^{-1}	3.83	2.43	3.06
Total P, g kg^{-1}	0.598	0.384	0.297

Road, 32%; Fort Sherman, 45%. These values did not change significantly during storage at 4, 22, or −35°C, and are not discussed further. Soil pH was determined in a 1:2 soil/solution ratio in both deionized water and 10 mmol L^{-1} CaCl$_2$ using a glass electrode.

Ammonium and NO$_3$ were determined by extraction in either 2 mol L^{-1} KCl (Albrook soil) or 0.5 mol L^{-1} K$_2$SO$_4$ (Pipeline Road and Fort Sherman soils). It was assumed that the N concentrations extracted by these two salt solutions were equivalent (Jones and Willett, 2006). Ammonium was determined by automated colorimetry at 660 nm following reaction with phenolate, while NO$_3$ was determined at 520 nm following Cd-catalyzed reduction to NO$_2$ and reaction with sulfanilamide at pH 8.5. Both NH$_4$ and NO$_3$ were determined using a Lachat Quickchem 8500 flow injection analyzer (Hach Co., Loveland, CO).

Readily exchangeable PO$_4$ was determined by extraction with an anion exchange membrane (AEM) using a method based on that described by Myers et al. (1999). Soil (5 g on a dry-weight basis) was shaken for 24 h with 80 mL of deionized water and five anion-exchange membrane strips (1 by 4 cm, manufactured by BDH, Poole, UK, and distributed by VWR International, West Chester, PA). The strips were rinsed in deionized water and the PO$_4$ recovered by shaking for 1 h in 50 mL of 0.25 mol L^{-1} H$_2$SO$_4$. Molybdate-reactive P was determined in the acid solution at 880 nm, following online neutralization with NaOH, by automated colorimetry using the Lachat Quickchem 8500. This is likely to represent mainly PO$_4$, but could also include small concentrations of acid-labile organic or condensed P compounds extracted by the membranes and hydrolyzed during elution in 0.25 mol L^{-1} H$_2$SO$_4$. For simplicity, we refer to this fraction as AEM-extractable PO$_4$.

Cations (Al, Ca, K, Mg, and Zn) were extracted from the Pipeline Road and Fort Sherman soils using a Mehlich-3 solution (Mehlich, 1984). Soil (5 g on a dry-weight basis) was shaken for 5 min in 50 mL of Mehlich-3 solution (0.2 mol L^{-1} NH$_4$OAc, 0.25 mol L^{-1} NH$_4$NO$_3$, 13 mmol L^{-1} HNO$_3$, 15 mmol L^{-1} NH$_4$F, and 1 m mol L^{-1} ethylenediaminetetraacetic acid). The solution was centrifuged (8000 × g for 10 min) and analyzed for cations and P by inductively coupled plasma optical-emission spectrometry (ICP–OES) using an Optima 2100 (PerkinElmer, Waltham, MA). To assess the proportions of inorganic and organic P in the Mehlich-3 extracts, molybdate-reactive P, assumed to be mainly PO$_4$, was determined at 880 nm by automated colorimetry with organic P
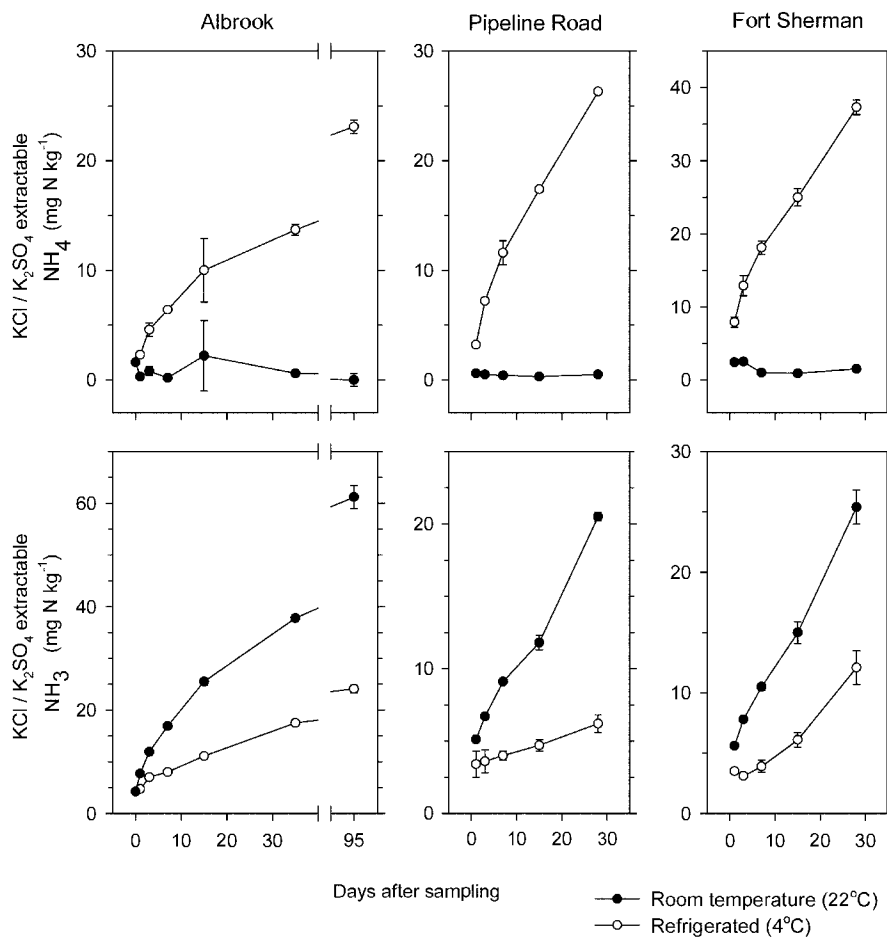
Fig. 1. Temporal changes in extractable NH_4 and NO_3 concentrations in three soils under lowland tropical rain forest in central Panama during storage at 4 and 22°C. The Albrook soil was extracted in 2 mol L^{-1} KCl, while the Pipeline Road and Fort Sherman soils were extracted in 0.5 mol L^{-1} K_2SO_4. Error bars show the standard deviation of four replicate samples.

determined as the difference between total P and molybdate-reactive P. Standards were prepared in the extraction solution for all analyses.

Determination of Soil Properties

Subsamples of each air-dried soil were ground in a ball mill and stored in sealed plastic bags at ambient laboratory temperature and humidity before determination of total elements and texture. Total C and N were determined by combustion and gas chromatography using a Thermo Flash NC1112 Soil Analyzer (CE Elantech, Lakewood, NJ). Total P was determined by ignition (550°C for 1 h) and extraction in 1 mol L^{-1} H_2SO_4 (1:50 soil/solution ratio, 16 h), with PO_4 detection by automated molybdate colorimetry using a Lachat Quickchem 8500. This procedure gave quantitative recovery of total P from a certified reference soil (Loam Soil D, High Purity Standards, Charleston, SC). The concentrations of sand (53 μm–2 mm), silt (2–53 μm), and clay (<2 μm) size particles were determined by the pipette method after pretreatment to remove organic matter (H_2O_2 oxidation), and Fe oxides (citrate–bicarbonate–dithionite reduction) (Gee and Or, 2002). Amorphous Al, Fe, Mn, and P were determined by extraction in a solution containing ammonium oxalate and oxalic acid (Loeppert and Inskeep, 1996) with detection by ICP–OES.

Statistical Analysis

Changes during storage at 4 and 22°C were determined by repeated measures analysis of variance using time and treatment as factors. Values

for fresh samples were compared with those from soils stored for 4 wk (room temperature, refrigerated, frozen, and air dried) by one-way analysis of variance with Tukey's honestly significant difference test for mean separation ($P < 0.05$). All statistical analysis was performed using R software (www.r-project.org; verified 20 July 2009).

RESULTS
Extractable Nitrogen
Ammonium

Ammonium was one of the most unstable nutrients, although changes varied markedly depending on the treatment. Concentrations declined rapidly to low values in all soils following storage at room temperature and stayed low throughout the remainder of the storage period (Fig. 1). In direct contrast, NH_4 concentrations in refrigerated samples increased continually ($P < 0.0001$ for both treatment and time effects for all soils). Even after overnight storage, NH_4 concentrations in extracts of the Pipeline Road and Fort Sherman soils stored at 4 and 22°C were significantly different. For example, extractable NH_4 in the Pipeline Road soil was 0.61 ± 0.12 mg N kg^{-1} when stored overnight at room temperature, but 3.18 ± 0.07 mg N kg^{-1} when refrigerated (Table 2), a more than five-fold difference. Frozen samples contained significantly greater NH_4 concentrations than fresh samples, while the effect of air drying differed among the three soils (Table 2), being higher (Albrook soil), lower (Fort Sherman soil), or not different (Pipeline Road soil) compared with fresh samples.

Nitrate

In contrast to NH_4, NO_3 concentrations increased continually following storage at both 4 and 22°C (time effect $P < 0.0001$ for all soils) (Fig. 1, Table 2), although samples stored at room temperature always contained a higher NO_3 concentration than refrigerated samples for a given time (treatment effect $P < 0.0001$ for all soils). The changes were rapid, with the concentration almost doubling in the Albrook soil during overnight storage at room temperature (the same change occurred after 3 d in refrigerated samples). Frozen storage of the Pipeline Road and Fort Sherman soils preserved NO_3 at similar concentrations to fresh samples (Table 2), but caused a marked decline for the acidic Albrook soil, indicating a possible effect of soil pH on NO_3 stability. (Note: NO_3 determined after 3 mo of storage in the Albrook soil) Air drying increased NO_3 concentrations compared with fresh samples for all soils (Table 2).

Anion-Exchange Membrane Extractable Phosphate

There were rapid changes in AEM-extractable PO_4 (time effect $P < 0.0001$ for all soils; Fig. 2). Refrigerated samples always contained lower AEM-extractable PO_4 values than those stored

at room temperature (treatment effect $P < 0.0001$ for all soils), while concentrations in both air-dried and frozen samples were markedly higher than in fresh samples (Table 2). There were differences among the soils, however, in terms of the storage conditions that best maintained AEM-extractable PO_4 concentrations close to field conditions. Room-temperature storage caused a decline in AEM-extractable PO_4 in the Albrook soil, from 2.81 ± 0.51 mg P kg^{-1} on the day of sampling to 1.09 ± 0.11 mg P kg^{-1} after 3 d, whereas concentrations in the Fort Sherman and Pipeline Road soils stored at room temperature were relatively stable for up to 4 wk (Fig. 2). In contrast, refrigerated samples of the Albrook soil were stable for at least 7 d, whereas concentrations in refrigerated samples of the Pipeline Road and Fort Sherman soils increased markedly after 24 h of storage (e.g., a four-fold increase for the Fort Sherman soil from 0.33 ± 0.08 to 1.33 ± 0.41 mg P kg^{-1}).

Mehlich-3 Extractable Cations and Phosphorus (Pipeline Road and Fort Sherman Soils Only)

The effects of storage treatment and time on extractable cations varied between the two soils and among the five elements. In particular, the changes following storage were much greater for the Fort Sherman soil than the Pipeline Road soil.

Aluminum concentrations changed significantly during refrigerated and room-temperature storage for both soils (time effect $P < 0.0001$; Fig. 3). For the Pipeline Road soil, there were no significant differences between samples stored at 4 or 22°C (treatment effect $P > 0.05$) and only refrigerated samples were significantly different from fresh samples after 4 wk of storage, with no significant differences for air-dried or frozen samples (Table 3). For the Fort Sherman soil, however, all treatments except freezing caused significant changes compared with fresh samples (Table 3).

Calcium and Mg concentrations were influenced by the duration of storage at 4 and 22°C only for the Sherman soil (time effect $P < 0.0001$), while K concentrations were influenced significantly for both soils (Fig. 3). For the Pipeline Road soil, only air drying significantly changed the concentrations of Ca and Mg compared with fresh samples, while K concentrations were not significantly different for any treatment (Table 3). For the Fort Sherman soil, storage at both 4 and 22°C significantly changed Ca and Mg concentrations compared with fresh samples, while only air-dried samples were significantly different for K (Table 3). The effects of air drying were, however, inconsistent, with a decrease in

Table 2. Storage effects on extractable inorganic N and P concentrations in three soils under lowland tropical rain forest in central Panama. For N fractions, the Albrook soil was extracted in 2 mol L^{-1} KCl, while the Pipeline Road and Fort Sherman soils were extracted in 0.5 mol L^{-1} K_2SO_4. Fresh samples were extracted either on the same day as sampling (Albrook) or after overnight storage at 4 or 22°C (Pipeline Road and Fort Sherman). All other treatments are for samples extracted after 4 wk of storage (5 wk for Ablrook). Values are the mean ± standard deviation of four replicate samples, and values within a column for each nutrient with the same letter are not significantly different at the 5% level (Tukey's honestly significant difference).

Ion and treatment	Albrook	Pipeline Road	Fort Sherman
NH_4, mg N kg^{-1}			
Fresh (same day)	1.56 ± 0.02 a		
Fresh (4°C overnight)		3.18 ± 0.07 a	7.94 ± 0.66 a
Fresh (22°C overnight)		0.61 ± 0.12 b	2.37 ± 0.21 b
Room temperature (22°C, 4 wk)	<0.01 b	0.54 ± 0.12 b	1.45 ± 0.14 b
Refrigerated (4°C, 4 wk)	13.71 ± 0.49 c	26.34 ± 0.18 c	37.31 ± 0.98 c
Frozen (−35°C, 4 wk)	4.68 ± 0.21 d	9.07 ± 0.47 d	12.01 ± 0.25 d
Air dried	6.09 ± 0.82 e	3.62 ± 0.28 a	5.07 ± 1.80 e
NO_3, mg N kg^{-1}			
Fresh (same day)	4.23 ± 0.16 a		
Fresh (4°C overnight)		3.39 ± 0.87 a	3.50 ± 0.15 a
Fresh (22°C overnight)		5.07 ± 0.14 b	5.65 ± 0.14 a
Room temperature (22°C, 4 wk)	37.83 ± 0.40 b	20.55 ± 0.28 c	25.41 ± 1.39 b
Refrigerated (4°C, 4 wk)	17.48 ± 0.36 c	7.77 ± 0.60 d	12.10 ± 1.41 c
Frozen (−35°C, 4 wk)	0.28 ± 0.17d†	3.94 ± 0.12 a	3.13 ± 0.10 b
Air dried	6.79 ± 0.21 e	6.19 ± 0.26 e	5.98 ± 1.85 a
PO_4 (mg P kg^{-1})‡			
Fresh (same day)	2.81 ± 0.51 a		
Fresh (4°C overnight)		2.11 ± 0.37 a	0.33 ± 0.08 a
Fresh (22°C overnight)		1.06 ± 0.29 a	0.13 ± 0.06 a
Room temperature (22°C, 4 wk)	1.17 ± 0.15 b	1.26 ± 0.05 a	0.23 ± 0.04 a
Refrigerated (4°C, 4 wk)	5.58 ± 0.09 c	4.75 ± 0.34 b	1.41 ± 0.23 b
Frozen (−35°C, 4 wk)	15.34 ± 1.34 d	5.82 ± 1.14 b	5.44 ± 0.69 c
Air dried	ND§	5.21 ± 1.27 b	4.92 ± 0.86 c

† Approximately 3 mo frozen storage.
‡ Determined by extraction with anion-exchange membranes.
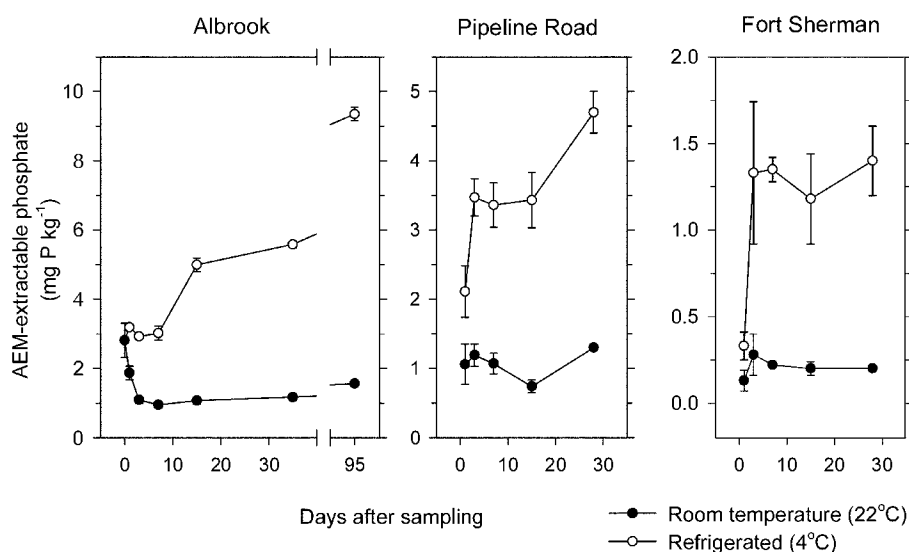§ ND, not determined.

Fig. 2. Temporal changes in PO_4 extracted by anion-exchange membrane (AEM) in three soils under lowland tropical rain forest in central Panama during storage at 4 and 22°C. Error bars show the standard deviation of four replicate samples.
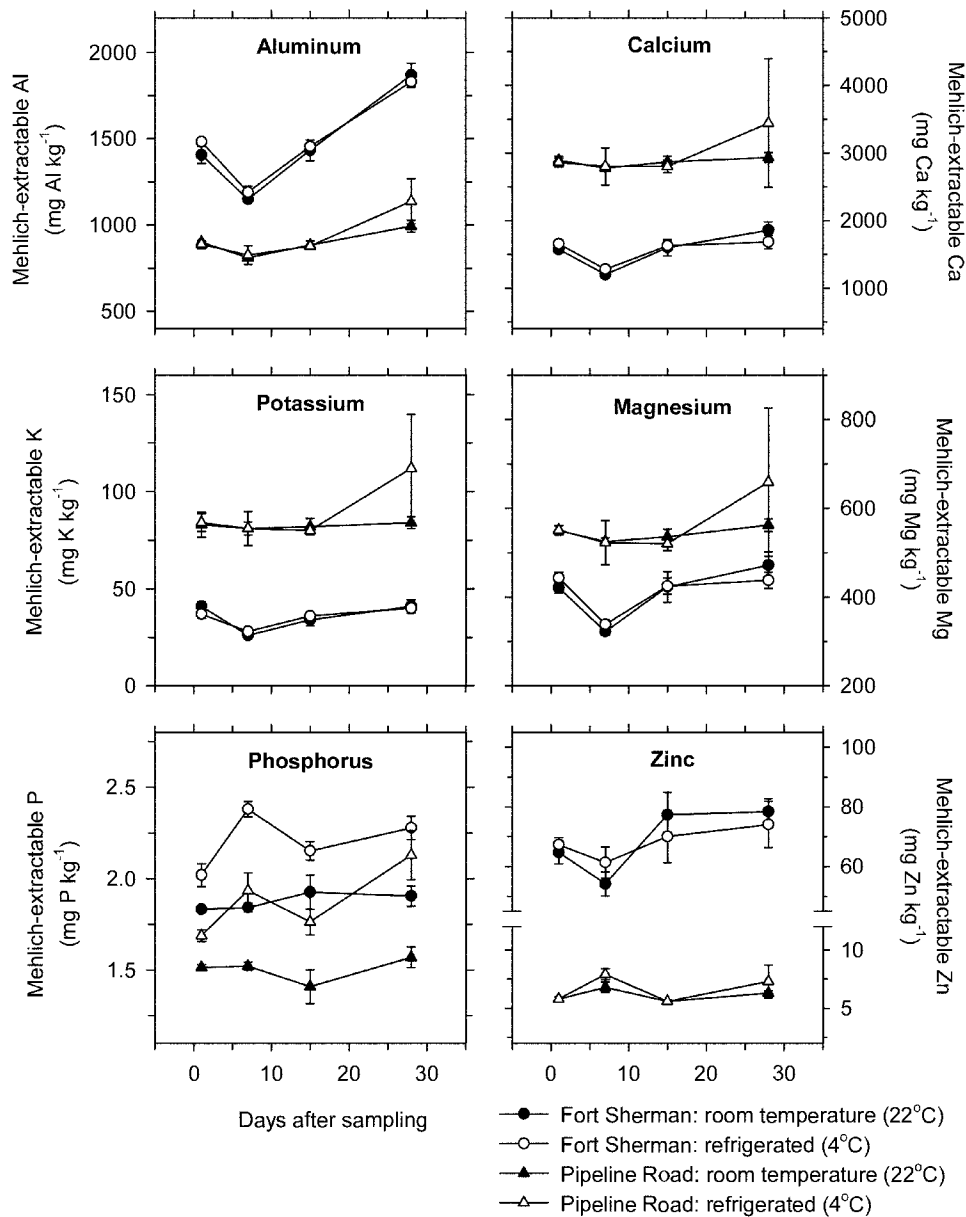
Fig. 3. Temporal changes in Mehlich-3 extractable cations (Al, Ca, K, Mg, and Zn) and total P in three soils under lowland tropical rain forest in central Panama during storage at 4 and 22°C. Error bars show the standard deviation of four replicate samples.

soil, whereas freezing and room-temperature storage caused an increase.

Soil pH

For all soils in both water and $CaCl_2$, the pH was lower when samples were stored at room temperature than when refrigerated (treatment effect $P < 0.0001$), even after only a short period of storage (Fig. 4). Values declined more or less continually during storage at both temperatures (time effect $P < 0.0001$ for all soils), with up to a 0.6 unit decline in pH in water after 3 mo storage of the Albrook soil at 4°C (Fig. 4). Relatively minor, although statistically significant, differences occurred following freezing and air drying (Table 4). Values tended to decrease following freezing, but the changes following air drying were inconsistent. For example, the pH of the Albrook soil in water increased following drying, but decreased for the other two soils (Table 4). Changes in dried soils were smaller when the pH was measured in 10 mmol L^{-1} $CaCl_2$ compared with water.

DISCUSSION

Recommendations for the storage of tropical forest soils for the determination of extractable nutrients are summarized in Table 5. Of the storage conditions assessed here, all resulted in marked changes in extractable inorganic nutrients. It therefore seems that for measurements that aim to approximate field conditions, extraction should be performed on the same day as, or within 24 h of, soil sampling. Analysis of fresh soil appears important even for soil pH, although analysis of refrigerated samples within 2 wk is probably acceptable.

The most sensitive measurements were of inorganic N fractions, which changed rapidly following sampling in all storage treatments. Similar results for NO_3 were reported during storage of temperate hardwood forest soils (both organic and mineral horizons of Spodosols) and occurred in parallel with declines in pH (Ross and Bartlett, 1990). Hart et al. (1994) recommended that for KCl extraction of NH_4 and NO_3, soil samples should be stored cool (2–5°C) and analyzed within 2 d of collection; alternatively, freezing was recommended to preserve inorganic N concentrations for subsequent analysis. This protocol has been followed in studies of tropical forest soils (e.g., Hall and Matson, 2003; Hedin et al., 2003), although it would clearly be inappropriate for the soils studied here.

K and Mg for the Pipeline Road soil and an increase for the Sherman soil (Table 3).

Total P concentrations in the Mehlich-3 extracts were approximately four times the PO_4 concentrations in the same extracts (data not shown), indicating the inclusion of a relatively large proportion of organic P in the values determined by ICP-OES. Concentrations were strongly influenced by treatment and time for both soils (Fig. 3), although after 4 wk of storage only refrigerated and frozen samples were significantly different from fresh samples (Table 3). Air drying had a limited effect for both soils.

Zinc concentrations were significantly influenced by storage time (time effect $P < 0.0001$), although the effects differed between the two soils. For the Pipeline Road soil, 4 wk of refrigerated storage caused a significant increase in extractable Zn compared with fresh samples, while drying caused a significant decease (Table 3). In contrast, drying had no effect on extractable Zn in the Fort Sherman

Based on our results, rapid extraction, preferably in the field or on the day of sampling, is essential to ensure that NH_4 concentrations approximate those in the field. For NO_3, cold storage and extraction within 48 h appears reasonable, but as NO_3 is usually extracted simultaneously with NH_4, extraction in the field or on the day of sampling is probably the only suitable procedure. Freezing preserved NO_3 concentrations in two soils, but the marked decline for the Albrook soil suggests that freezing is unsuitable for very acidic soils. Rapid air drying may be an option for longer term storage of NH_4 and NO_3: here we used 10 d at room temperature, but drying for a shorter period of time at a higher temperature (e.g., 35°C) may preserve NH_4 concentrations at values similar to those in the field. In this case, drying should begin as soon as possible following sampling.

Readily extractable PO_4 was also sensitive to storage conditions, although some of the effects appeared to be soil specific. This seems linked to soil pH, which was previously reported to influence $CaCl_2$–extractable PO_4 following storage of temperate soils from northern England (Gupta and Rorison, 1974), for which values increased in acidic soils, but not calcareous soils, stored at field moisture and ambient laboratory temperature. Given the unpredictable changes that can occur in extractable PO_4 during short-term storage, we recommend that samples be analyzed fresh within 24 h of collection, although additional information on a larger number of soils might allow more detailed recommendations based on soil properties. Air drying, freezing, and long-term refrigeration were clearly unsuitable, although long-term storage (up to 3 mo) at ambient laboratory temperature may be acceptable for soils of pH >5.

Chapman et al. (1997) also reported that soil storage before separation of the soil solution by centrifugation caused a marked decline in P concentrations, including those of molybdate-reactive P and dissolved organic P, within a few days. Changes in extractable P following air drying are well known, and can occur for both inorganic and organic P fractions (Sparling et al., 1985; Turner and Haygarth, 2001, 2003), including resin-extractable PO_4 (Olsen and Court, 1982). For a series of Inceptisols and Alfisols under permanent pasture in the UK, the greatest changes in $NaHCO_3$–extractable PO_4 following 7 d of drying at 30°C occurred in acidic, low-P soils (Turner and Haygarth, 2003), properties which occur commonly in soils under lowland tropical forests. The changes following drying have been linked to direct effects on microbes (Sparling et al., 1985; Turner et al., 2003), as well as the disruption of aggregates and organic matter coatings on clay minerals (Nevo and Hagin, 1966; Bartlett and James, 1980). Drying also influences PO_4 sorption onto Al and Fe oxides (McLaughlin et al., 1981; Haynes and Swift, 1989), which is likely to have relevance for strongly weathered tropical forest soils.

Extractable cations (Ca, K, and Mg) were less influenced by storage than N and P fractions, although the effects varied between the two soils studied. Extraction of fresh samples following no more than 7 d of field-moist storage is therefore preferable to prolonged storage if an approximation of field conditions is required. For extractable K, Gupta and Rorison (1974) reported that $CaCl_2$–extractable K concentrations were lowest in refrigerated samples of both acidic and calcareous soils from northern England, with much greater values for the calcareous soils when stored at ambient laboratory temperature. For the acidic soils, air drying caused a greater increase in extractable K than storage at laboratory temperature.

Table 3. Storage effects on cation (Al, Ca, K, Mg, and Zn) and total P concentrations in Mehlich-3 extracts of three soils under lowland tropical rain forest in central Panama. Fresh samples were extracted after overnight storage at 4 or 22°C, with other treatments extracted after 4 wk of storage. Values are the mean ± standard deviation of four replicate samples, and values within a column for each nutrient with the same letter are not significantly different at the 5% level (Tukey's honestly significant difference).

Element and treatment	Pipeline Road	Fort Sherman
Al, mg kg⁻¹		
Fresh (4°C overnight)	888 ± 24 a	1482 ± 9 a
Fresh (22°C overnight)	901 ± 6 a	1406 ± 49 ab
Room temperature (22°C, 4 wk)	994 ± 35 ab	1868 ± 70 c
Refrigerated (4°C, 4 wk)	1138 ± 130 bc	1830 ± 28 c
Frozen (−35°C, 4 wk)	976 ± 35 a	1562 ± 27 ab
Air dried	942 ± 80 a	1981 ± 303 d
Ca, mg kg⁻¹		
Fresh (4°C overnight)	2860 ± 67 a	1655 ± 54 a
Fresh (22°C overnight)	2891 ± 63 a	1571 ± 43 a
Room temperature (22°C, 4 wk)	2934 ± 73 a	1857 ± 125 b
Refrigerated (4°C, 4 wk)	3445 ± 954 a	1682 ± 96 ac
Frozen (−35°C, 4 wk)	2889 ± 129 a	1834 ± 56 bc
Air dried	2004 ± 629 b	1855 ± 65 bd
K, mg kg⁻¹		
Fresh (4°C overnight)	84 ± 5 abc	37 ± 2 a
Fresh (22°C overnight)	83 ± 7 ab	41 ± 2 ab
Room temperature (22°C, 4 wk)	84 ± 3 abc	41 ± 3 ab
Refrigerated (4°C, 4 wk)	112 ± 28 c	40 ± 3 ab
Frozen (−35°C, 4 wk)	101 ± 9 ac	45 ± 1 b
Air dried	75 ± 14 b	53 ± 6 c
Mg, mg kg⁻¹		
Fresh (4°C overnight)	551 ± 9 a	443 ± 13 a
Fresh (22°C overnight)	550 ± 11 a	421 ± 12 a
Room temperature (22°C, 4 wk)	562 ± 14 a	472 ± 30 ab
Refrigerated (4°C, 4 wk)	659 ± 167 a	438 ± 18 a
Frozen (−35°C, 4 wk)	562 ± 21 a	484 ± 14 b
Air dried	427 ± 89 b	505 ± 20 b
P, mg kg⁻¹		
Fresh (4°C overnight)	1.69 ± 0.03 a	2.02 ± 0.06 a
Fresh (22°C overnight)	1.51 ± 0.03 a	1.83 ± 0.02 b
Room temperature (22°C, 4 wk)	1.57 ± 0.01 a	1.90 ± 0.06 ab
Refrigerated (4°C, 4 wk)	2.13 ± 0.13 b	2.28 ± 0.06 c
Frozen (−35°C, 4 wk)	2.37 ± 0.17 b	3.53 ± 0.16 d
Air dried	1.51 ± 0.15 a	1.78 ± 0.01 b
Zn, mg kg⁻¹		
Fresh (4°C overnight)	5.8 ± 0.2 ab	67 ± 2 ab
Fresh (22°C overnight)	5.8 ± 0.2 ab	65 ± 4 a
Room temperature (22°C, 4 wk)	6.3 ± 0.2 ac	78 ± 4 bc
Refrigerated (4°C, 4 wk)	7.3 ± 1.4 c	74 ± 8 abc
Frozen (−35°C, 4 wk)	6.4 ± 0.2 ac	86 ± 10 c
Air dried	4.6 ± 0.2 b	73 ± 1 abc

Of course, in many cases it will clearly be difficult to process tropical soils rapidly after sampling, particularly when working in remote locations or when samples must be transported some distance to laboratory facilities. In such circumstances, the most appropriate procedure may be to extract samples in the field (e.g., Ross et al., 2006; Hales and Ross, 2008), using preweighed bottles to which the extraction solution is added in advance. The solu-
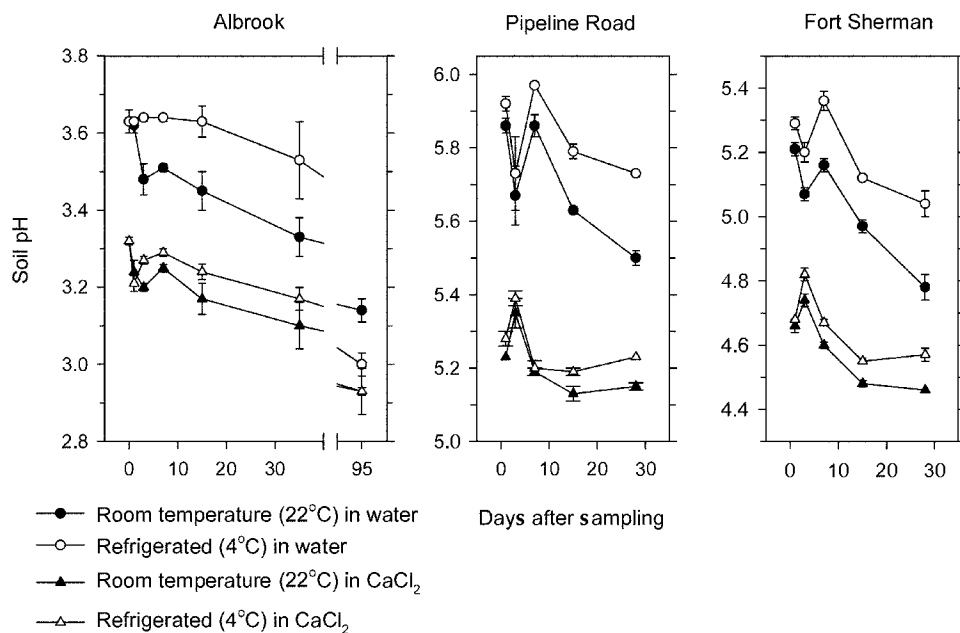
Fig. 4. Changes in soil pH determined in deionized water and 10 mmol L⁻¹ CaCl₂ in three soils under lowland tropical rain forest in central Panama during storage at 4 and 22°C. Error bars show the standard deviation of four replicate samples.

Fig. 4. Changes in soil pH determined in deionized water and 10 mmol L^{-1} $CaCl_2$ in three soils under lowland tropical rain forest in central Panama during storage at 4 and 22°C. Error bars show the standard deviation of four replicate samples.

Table 4. Storage effects on pH determined in either deionized water or 10 mmol L⁻¹ CaCl₂ in three soils under lowland tropical rain forest in central Panama. Fresh samples were extracted either on the same day as sampling (Albrook) or after overnight storage at 4 or 22°C, with other treatments extracted after 4 wk of storage(5 wk for Albrook). Values are the mean ± standard deviation of four replicate samples, and values within a column for either determination in water or CaCl₂ with different letters are significantly different at the 5% level (Tukey's honestly significant difference).

Treatment	Albrook	Pipeline Road	Fort Sherman
Soil pH (H₂O)			
Fresh (same day)	3.63 ± 0.03 a		
Fresh (4°C overnight)		5.92 ± 0.02 a	5.29 ± 0.02 a
Fresh (22°C overnight)		5.86 ± 0.02 b	5.21 ± 0.02 b
Room temperature (22°C, 4 wk)	3.33 ± 0.10 b	5.50 ± 0.02 c	4.78 ± 0.04 c
Refrigerated (4°C, 4 wk)	3.53 ± <0.01 a	5.73 ± 0.01 d	5.04 ± 0.04 d
Frozen (−35°C, 4 wk)	3.80 ± <0.01 c	5.74 ± <0.01 d	5.07 ± 0.02 d
Air dried	3.77 ± 0.04 c	5.84 ± <0.01 b	4.98 ± 0.01 e
Soil pH (CaCl₂)			
Fresh (same day)	3.32 ± 0.01 a		
Fresh (4°C overnight)		5.28 ± 0.02 a	4.68 ± <0.01 a
Fresh (22°C overnight)		5.23 ± <0.01 b	4.66 ± 0.02 a
Room temperature (22°C, 4 wk)	3.10 ± 0.06 b	5.15 ± 0.01 c	4.46 ± <0.01 b
Refrigerated (4°C, 4 wk)	3.17 ± 0.03 bc	5.23 ± <0.01 b	4.57 ± 0.02 c
Frozen (−35°C, 4 wk)	3.23 ± 0.04 c	5.24 ± 0.02 b	4.65 ± 0.01 cd
Air dried	3.36 ± 0.04 a	5.35 ± 0.01 d	4.57 ± 0.04 ae

Table 5. Recommended storage conditions for the determination of extractable nutrients and soil pH representative of field conditions in tropical forest soils.

Measurement	Recommended analysis time	Long-term storage	Not recommended
NH₄	Fresh (<6 h)	None	Any form of storage
NO₃	Fresh (<6 h)	Frozen (soil pH > 5)	Room temperature, 4°C > 7 d, frozen (soil pH < 5)
PO₄ by anion exchange membrane	Fresh (<4 h)	4 wk at room temperature (soil pH > 5)	Frozen, air dried, 4°C > 7 d
Mehlich-3 P	Fresh (<24 h)	4 wk at room temperature (soil pH > 5)	4°C > 7 d
Cations (Ca, K, Mg)	Fresh (<24 h)	4 wk at 4°C	
Soil pH	Fresh (<24 h)	2 wk at 4°C	Room temperature

tion can be later decanted and stabilized before transport to the location of analysis and the weight of the soil can be calculated by drying the bottle, although it should be noted that changes in NH_4 concentrations can still occur (Ross et al., 2006). For PO_4, the anion-exchange membranes can be removed after field extraction and stored in deionized water until subsequent processing.

An alternative procedure, although requiring multiple visits to the same site, is the use of ion-exchange resins (both cation and anion) installed in the soil (e.g., Crews et al., 1995; Yavitt and Wright, 1996; Meason and Idol, 2008). These have proved useful in comparing nutrient availability among similar soils (e.g., Vitousek, 2004), especially as they yield an integrated measure of nutrient availability during the time that they are in the field. There are several disadvantages, however, associated with the use of ion-exchange resins in the field (discussed in Meason and Idol, 2008), not least that they do not yield a quantitative estimate of nutrient concentrations, on either a mass or volume basis.

Finally, transformations of extractable nutrients, particularly inorganic N fractions, are well known (e.g., Munro and Mackay, 1964; Ross and Bartlett, 1990; Hart et al., 1994), yet it is often unclear in the published literature how samples were stored and how much time passed between sampling and extraction (e.g., Yavitt and Wright, 1996; Yamashita et al., 2003; Davidson et al., 2004). Recognizing that rapid extraction is not possible in most cases, but that differences in storage time are likely to influence the results, we recommend that the time between sampling and extraction be reported as carefully as possible, including the conditions under which samples were stored in the interim period. This will help considerably in interpreting data from various studies.

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