NOTE

Freezing Low Volume Aqueous Solutions to Preserve Ammonia and Nitrate plus Nitrite

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ABSTRACT

Analysis of N and P in leachate is important in studying the fate of fertilizers after they are applied to turfgrass and landscape plants. Depending on environmental factors, in situ leachate samples collected from the field are often less than 20 mL. Due to the time and resource commitment of N and P analysis, it is desirable for many samples to be collected before analysis is conducted. In addition, past research has documented that acid preservation of low volume samples for N analysis can lead to inaccurate P measurements. Two experiments conducted in June 2007 and August 2008 compared ammonia-nitrogen (NH_3–N) and nitrite-nitrogen plus nitrate-nitrogen (NO_2–N + NO_3–N) concentrations within low volume samples preserved by freezing to other commonly used preservation techniques, including the accepted USEPA method. All preservation methods, including storage at less than –20°C, resulted in NH_3 and NO_2 + NO_3 concentrations similar to concentrations measured in immediately analyzed aliquots. Freezing low volume leachate water samples for later N analysis is an adequate method of preservation.

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Abbreviations: ANOVA, analysis of variance; MDL, method detection limit; PQL, practical quantification limit.

Identifying the environmental fate of N and phosphorus P applied to turfgrass systems is important (Frank et al., 2006). One pathway for N and P loss is water percolating past the plant root zones (Paré et al., 2008; Wherley et al., 2009; Erickson et al., 2010). Nitrogen and P that remains in porewater after passing through the rootzone have the potential to contaminate groundwater. Due to the large number of plots and cost of equipment, ceramic cup samplers and large drainage lysimeters tend to be the preferred equipment for extracting soil solution to monitor quantity and quality in native soils (Barbarick et al., 1979). Consistency of sampler installation and sample collection methods, percolate water chemistry, the volume collected, and analysis methods are all sources of variability (Hansen and Harris, 1975; Harris and Hansen, 1975; Levin and Jackson, 1977; Debyle et al., 1988). When researching the movement of water through soil in landscapes and turfgrasses, collection of very low volumes of leachate (≤20 mL) are common. Under such circumstances, all leachate should be collected, with no loss to rinsing collection bottles (which is strongly encouraged), ensuring adequate sample volume for analysis.

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The current guideline for NO_2–N + NO_3–N preservation (USEPA Method 353.2) recommends refrigeration at 4°C, if analyzed within 24 h and preservation with 2 mL sulfuric acid (H_2SO_4) per liter and refrigeration if analysis will not be within 24 h. Guidelines for NH_3–N (USEPA Method 350.1) samples are to be preserved with 2 mL H_2SO_4 per liter and refrigerated at 4°C and can only be held up to 24 d (USEPA, 1983a, 1983b). Klingaman and Nelson (1976) investigated storage methods to preserve inorganic N for up to 12 wk from unfiltered, surface, tile drain, and stream water samples and determined that storage at subzero temperature was as effective as 4°C samples preserved with phenylmeruric acetate ($C_8H_8HgO_2$) or mercury chloride ($HgCl_2$) in lieu of H_2SO_4 (to prevent any N loss from oxide formation). Degobbis (1973) reported that immediate storage in subzero temperatures is the best method for preservation of NH_3–N in seawater samples compared with chemical preservation with phenol (C_6H_6O). Proctor (1962) compared freshly collected unpreserved seawater to samples frozen for more than 220 d and the frozen samples matched the baseline analysis within the precision limits of the analytical method. Avanzino and Kennedy (1993) documented that freezing was an effective preservation method for NO_2–N + NO_3–N in samples out to 8 yr with a 95% confidence level. Acidification for N preservation would require adding small quantities of H_2SO_4 to low volume samples and could interfere with the analysis of phosphorus (Kotlash and Chessman, 1998). Measuring nutrient concentrations in leachate is resource (time, labor, equipment, chemical, and money) consuming. It is sometimes a more efficient use of resources to analyze samples once a larger number of samples have accumulated. Thus, it is important to determine a reliable and simple preservation process that ensures sample integrity without compromising sample N concentrations for field-collected, low volume samples. Two studies were conducted with the objective to identify adequate preservation techniques that would not compromise the concentration of NO_2–N + NO_3–N, and NH_3–N detected in low volume (≤20 mL) leachate samples.

MATERIALS AND METHODS

Percolate samples were collected on 4 June 2007 from 29 ceramic cup samplers, installed in a Bonneau soil (Loamy, siliceous, subactive, thermic Arenic Paleudult) below the root zone (40-cm deep) of 3.0 × 3.2-m bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] plots maintained under golf course fairway conditions at the Clemson University Pee Dee Research and Education Center in Florence, SC. Leachate was collected using a vacuum maintained at 0.03 MPa. Upon collection, a 5-mL aliquot was immediately analyzed (immediate) with the remainder of the sample split into four 5-mL aliquots, each placed into 20-mL polyethylene scintillation vials and then preserved and stored in one of four ways: held at room temperature (24°C) and analyzed 20 h after collection (next day); refrigerated at 4°C and analyzed 24 h after collection (refrigerated); frozen at –20°C and analyzed 11 d after collection (frozen); and acidified to a pH of 2 using H_2SO_4 and refrigerated at 4°C, then analyzed 11 d after collection (USEPA Method 353.2; USEPA, 1983a). Samples were immediately placed in the appropriate refrigerator or freezer compartment of a side-by-side Kenmore Frostfree 20 unit (Sears, Illinois, USA). Frozen samples were placed in a room temperature (24°C) water bath until thawed. Samples were colormetrically analyzed by a Lachat QuikChem 8000 following QuikChem method 30-107-06-1-A for NH_3–N (applicable range is 0.10 to 20.0 mg L^{-1}, practical quantification limit [PQL] = 0.11 mg L^{-1}, method detection limit [MDL] = 0.02 mg L^{-1}) and QuikChem method 10-107-04-1-J for NO_2–N + NO_3–N (applicable range is 0.10 to 5.00 mg L^{-1}, PQL = 0.03, MDL = 0.13) based on USEPA methods 350.1 and 353.2, respectively (USEPA 1983a; 1983b). Quality control and assurance was maintained by a matrix blank and standard every ten samples. The calibration curve was rerun if the matrix blank or standard failed. The experiment was conducted again on 11 Aug. 2008 using only six of the same ceramic cup samplers due to the fact that other samplers were in use for an experiment. In addition, freezing was the only preservation method compared with immediate analysis since it was the one of most interest. A completely randomized experimental design was used with the percolate sample collected from each ceramic cup sampler serving as a replication (32 and 6 replications for the 2007 and 2008 experiments, respectively).

The statistical analysis for the effect of storage preservation on nutrient concentration was conducted in two steps using SAS software (SAS, 2003). The first step involved determining if the nutrient concentration being above or below the MDL was due to storage preservation. This was determined by a generalized linear model using binominal distribution. No treatments significantly increased or decreased the proportion above or below the MDL. The second step involved the observations above the MDL and substituting all values that fell below the MDL with the MDL value using traditional analysis of variance (ANOVA) techniques to determine treatment effects. No differences were found. Assumptions for the ANOVA techniques (normal distribution, equal variances) were evaluated and found to not be an issue. Therefore, ANOVA means were reported for easy interpretation and data presentation. Treatment means were compared with immediate means by Dunnett's test at $\alpha = 0.05$.

RESULTS

In general, NH_3–N concentrations were higher than anticipated for leachate from turfgrass, ranging from 2.17 to 3.75 mg L^{-1} in 2007 and 0.02 to 14.2 mg L^{-1} in 2008. In 2007 and 2008, all preservation techniques were adequate for storing samples for NH_3–N ($p = 0.8727$) and NO_2–N + NO_3–N ($p = 0.9989$) analysis (Table 1). Although all preservation techniques were adequate, it is noteworthy that the acidification method (USEPA method) resulted in the greatest standard deviation and difference of means from the immediately analyzed aliquots (Table 1).

Table 1. Comparison of nitrogen (NH_3 and NO_2 + NO_3) preservation techniques to immediately analyzed samples examined in low volume leachate samples collected in 2007 and 2008 from managed turfgrass plots.

Analyte	Control and storage treatment	Mean	Std. dev.†	Diff. of means‡	*P* value
		(mg L^{-1})			
2007					
NH_3	Immediate	2.39	5.41	na§	na
	Next day	2.47	5.61	0.08	1.00
	Refrigerated	2.64	6.06	0.25	1.00
	Frozen	2.17	5.60	−0.22	1.00
	USEPA	3.75	7.24	1.36	0.99
NO_2 + NO_3	Immediate	2.19	2.97	na	na
	Next day	2.23	3.03	0.04	1.00
	Refrigerated	2.28	3.02	0.09	0.99
	Frozen	2.21	2.97	0.02	1.00
	USEPA	2.42	3.33	0.23	0.99
2008					
NH_3	Immediate	2.44	5.76	na	na
	Frozen	2.39	5.59	−0.05	0.91
NO_2 + NO_3	Immediate	1.93	1.34	na	na
	Frozen	2.35	1.32	0.42	0.58

† Standard deviation of the mean analyte concentration for each treatment.

‡ The difference in the mean analyte concentration of each treatment from the immediate mean. Treatment means were compared with immediate mean by Dunnett's test at $\alpha = 0.05$.

§ Not applicable.

DISCUSSION

Other researchers (Proctor, 1962; Avanzino and Kennedy, 1993; Degobbis, 1973; Klingaman and Nelson, 1976) have identified freezing as a suitable method for N preservation in both fresh water and seawater, where sample volume was not an issue. The results of this study suggest that subzero sample storage is an effective preservation method for stabilizing dissolved NH_3–N, NO_2–N + NO_3–N in low volume aqueous solutions. Stored samples preserved for later analysis should be consistently maintained at temperatures −20°C. Not having the H_2SO_4 within the sample eliminates the interference associated with the acid for P analysis. Although not statistically supported, it is of particular interest that the USEPA preservation method resulted in concentrations most different than those that were immediately analyzed (Table 1). This was unexpected as method 353.2 is still the currently accepted handling method (USEPA, 1983a). It is important to note the preliminary nature of this research due to the small sample size and narrow range of NH_3–N and NO2-N + NO_3–N concentrations. Further validation of using freezing as an adequate NH_3–N and NO_2–N + NO_3–N preservation method and validation of existing preservation methods should consider using other freezing temperatures, a larger sample size, and samples with a wider NH_3–N and NO_2–N + NO_3–N concentration range (perhaps by spiking samples with known quantities). In addition, as poorer quality alternative water sources become more commonly used for irrigation purposes, it may be necessary to re-evaluate freezing as a preservation technique for low volume leachate samples, with special attention to how quickly samples are frozen. Particulate matter, including dissolved organic matter and fine minerals, high salts, and microbes, can be present in elevated amounts in poorer quality water sources that can quickly change N forms present.

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