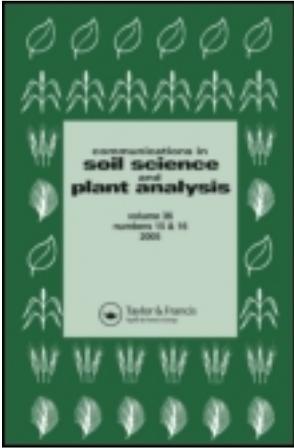


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Delayed Sample Filtration and Storage Effects on Dissolved Nutrients Measured in Agricultural Runoff

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Few research studies have examined the influence of delayed filtration on sample stability or runoff nutrient loss assessments. Runoff samples from irrigation furrows were each split into four volumes: two were filtered (45 μm) in the field and two were filtered 10 days later, with or without boric acid treatment, and stored at 4 °C. Sample dissolved reactive P (DRP), nitrate nitrogen ($\text{NO}_3\text{-N}$), and ammonium ($\text{NH}_4\text{-N}$) concentrations were measured in all filtered samples 10 and 107 days after collection. Samples filtered in the field and those with a 10-day delayed filtration had similar dissolved DRP, $\text{NO}_3\text{-N}$, and $\text{NH}_4\text{-N}$ concentrations, whether or not boric acid was added. Boric acid stabilized DRP and $\text{NH}_4\text{-N}$ sample concentrations, but not $\text{NO}_3\text{-N}$, during the 107 days of storage (relative to field-filtered samples). The effect of treatments on computed furrow stream concentration and runoff mass losses was similar to that for sample concentrations, except that furrow $\text{NH}_4\text{-N}$ parameters were unaffected by treatments. The field-filtered or 10-d delayed filtration without boric acid treatments provided the best dissolved nutrient measurements for comparing agricultural management effects at the field edge; however, results suggest that an incubation-type test for field-edge runoff water may provide a more accurate estimate of field management effects on dissolved nutrient loads in downstream aquatic ecosystems.

Keywords Dissolved P, inorganic N, runoff, sampling protocol, water quality

Introduction

Increased concentrations of dissolved reactive phosphorus (DRP), nitrate nitrogen ($\text{NO}_3\text{-N}$), and ammonium ($\text{NH}_4\text{-N}$) in runoff have been linked with the accelerated eutrophication of natural receiving waters (Levine and Schindler 1989). Researchers collect runoff water samples and analyze them for these nutrients to estimate the runoff's potential effect on aquatic ecosystems. Because they are biologically active, dissolved phosphorus (P) and nitrogen (N) concentrations in collected waters can change with time (Nelson and Römken 1972; Klingaman and Nelson 1976; Fishman, Schroder, and Shockey 1986; Kotlash and Chessman 1998). Dissolved P concentrations can also change in the presence of sediment in response to sorption or desorption reactions (Bjorneberg et al. 2006).

To ensure that sample nutrient concentrations accurately reflect those present at the time of sampling it is recommended that analyses be conducted immediately on fresh water

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samples (Robards et al. 1994). However, when this is not possible, the immediate storage of samples at 4 °C may suffice for 1 to 2 week storage times, particularly if samples do not contain substantial particulate concentrations (Fishman, Schroder, and Shockey 1986; Robards et al. 1994). Storing samples at -20 °C often stabilizes inorganic N concentrations but tends to destabilize dissolved P (Nelson and Römken 1972; Johnson, Bouldin, and Hergert 1975; Klingaman and Nelson 1976). Dissolved P concentrations in collected runoff water samples are appreciably stabilized if particulates are immediately removed, either by filtering (45 μm) or centrifugation (Klingaman and Nelson 1976; Lambert, Maher, and Hogg 1992; Kotlash and Chessman 1998). Biocide (HgCl_2) additions to refrigerated samples have also been recommended (Fishman, Schroder, and Shockey 1986) but are not frequently used due to environmental considerations (Robards et al. 1994). Biocides prevent utilization of nutrients by microorganism and plankton during sample storage (Thayer 1970) but the death of microorganisms present in water samples can result in nutrient increases (Fitzgerald and Faust 1967). The objectives of this study were to (1) examine the combined effect of delayed filtration and storage, with or without boric acid biocide, on the stability of dissolved nutrient concentrations in water samples and (2) determine how these sample treatments influence the calculated nutrient losses from irrigation runoff.

Materials and Methods

Site and Soils

Irrigation runoff water samples were collected from a furrow irrigation on a Portneuf silt loam (coarse-silty, mixed superactive, mesic Durinodic Xeric Haplocalcids) with 1.5% slope near Kimberly, Idaho, USA. The surface soil is a silt loam and contains on average 100 g kg^{-1} clay, 700 g kg^{-1} silt, 10 to 13 g kg^{-1} organic carbon, and 5% calcium carbonate equivalent. The soil has a saturated-paste-extract electrical conductivity (EC) of 0.07 S m^{-1} ; exchangeable sodium percentage (ESP) of 1.5; and pH of 7.7 (saturated paste). The Snake River water used for irrigation had an average EC of 0.05 S m^{-1} , pH of 7.9, and sodium adsorption ratio of 0.6 and carried little sediment (<500 mg L^{-1}).

Experimental Design

Twenty-eight runoff water samples were collected during the course of irrigation from the inflow, mid-field, and outflow locations of three randomly selected irrigation furrows. Each water sample was split, subject to eight different sample handling and storage treatments, and analyzed for dissolved $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and DRP. The measured sample nutrient concentrations from the different treatment procedures (inflow and outflow samples only) were then used to derive eight individual estimates for the average furrow runoff concentration and cumulative mass loss for each nutrient.

Sample Handling and Storage Treatments

Each of the 28 water samples collected from irrigation furrows were divided and subject to four different filtering and biocide treating protocols: (1) filtered (45 μm) immediately after collection with no biocide added and stored at 4 °C for 10 days; (2) filtered immediately after collection with biocide added (1 mL saturated boric acid solution per 100

mL of water sample) and stored at 4 °C for 10 days; (3) stored untreated sample at 4 °C for 10 days and then filtered; and (4) treated sample with biocide immediately after collection, stored at 4 °C for 10 days, and then filtered. On day 10 after collection all filtered samples from each treatment were subsampled and analyzed for NO₃-N, NH₄-N, and DRP. Samples were returned to cold storage (4 °C) and analyzed again on day 107. Thus the experiment consisted of eight treatments (2 filter times × 2 biocide levels × 2 storage times = 8). The experimental determination of treatment effects on individual sample nutrient concentrations included the 28 water samples as replicates, whereas that for treatment effect on furrow stream concentrations and mass losses included the three furrows as replicates.

Sampling and Analysis

The 12-h irrigation was started at 0900 h. Inflows were initially set to 22 L min⁻¹ and turned down to 15 L min⁻¹ after irrigation streams had advanced to the end of the field and runoff had begun. Furrow inflow rates, furrow stream outflow rates, and sediment concentrations were measured during the irrigation. Outflow rates were measured at one-half-hour intervals early in the irrigation, every hour during the mid-irrigation period, and every 2 h thereafter, when irrigation outflows and sediment loads had stabilized (typically after 7 h or more into the set). We collected water samples from small flumes in the irrigation streams, placed at the middle and end of three different furrows. Samples were collected at four times during the irrigation: 5 min, 30 min, 2 h, and 7 h after the stream runoff began at the mid- and end-furrow locations. In addition, irrigation inflow water was sampled at the same four times as the furrow.

Immediately after collection, each water sample was mixed thoroughly then split. One half of the sample was filtered through 0.45-micron Millipore¹ membrane and half was left unfiltered. Each of the filtered and unfiltered portions were split again, and one half was treated with a saturated boric acid (H₃BO₃) solution (1 mL per 100 mL sample) and the other half left untreated. All samples were immediately stored at 4 °C. Thus, we collected a total of 112 water samples (seven furrow locations, including inflow) × 4 times × 4 sample treatments. Ten days after collection all samples not filtered in the field were filtered in the laboratory using the same procedure as for the field-filtered samples. All samples were then analyzed for DRP (Watanabe and Olsen 1965) and NO₃-N and NH₄-N using flow injection analysis and colorimetric methods (Mulvaney 1996). All samples were then returned to storage at 4 °C for 97 days, after which they were reanalyzed (107 days after samples were collected) for the same dissolved nutrients evaluated previously.

Calculations and Statistical Analysis

The computer program WASHOUT calculated net infiltration and runoff volumes, mean nutrient concentrations, and runoff nutrient losses for furrows (Lentz and Sojka 1995). The program computed inflow and outflow volumes by integrating the inflow- and outflow-rate curves over time. Nutrient concentrations measured in runoff water samples and outflow rates were used to calculate nutrient loads in furrow stream outflows. Cumulative DRP, NO₃-N, and NH₄-N mass losses per irrigation were computed with the assumption that runoff constituent concentrations remained constant between sampling intervals. Reported

¹Manufacturer or trade names are included for the readers' benefit. The USDA-ARS neither endorses nor recommends such products.

nutrient concentrations and values used in mass-loss computations were adjusted for inflow concentrations, so furrow losses represented only those losses resulting from the furrow irrigation. These calculations were done using measured nutrient concentrations from each of the eight sample handling/storage treatments.

The sampled waters included a range of dissolved nutrient concentrations. These values were analyzed using a completely randomized design via analysis of variance (ANOVA), PROC Mixed (SAS Institute, Inc. 2009). The model included treatment as the fixed effect. When the fixed effect was significant, treatment means were separated using the Tukey option (SAS Institute, Inc. 2009). Runoff nutrient concentrations for samples were transformed using common Log to stabilize variances and improve normality.

An ANOVA was also conducted using PROC Mixed to determine the effect of treatment on each the two computed runoff nutrient parameters, the mean irrigation runoff concentration and cumulative nutrient loss for DRP, NO₃-N, and NH₄-N. The model included treatment as the fixed effect. When appropriate, response values were transformed as indicated previously.

Finally, Pearson's correlation evaluated whether sediment concentration influenced the difference in concentration between short vs. extended storage samples (day 107 minus day 10). This was evaluated using PROC CORR (SAS Institute 2009) and was run separately for lab-filtered only, lab-filtered + boric acid, field-filtered only, and field-filtered + boric acid treatment groups.

Results and Discussion

Treatments strongly influenced dissolved NO₃-N and DRP concentrations in runoff water samples, the calculated mean furrow stream values, and the cumulative furrow nutrient losses (Table 1). This contrasted with results for dissolved NH₄-N, where treatments

Table 1

Analysis of variance showing significance ($P > F$) of treatment effects on dissolved N and P nutrient concentrations for irrigation runoff samples, the calculated mean nutrient concentrations of irrigation furrows, and the cumulative nutrient losses of irrigated furrows

Source	<i>P</i> value		
	NO ₃ -N	NH ₄ -N	DRP
Sample concentration			
Treatment (T)	***	**	***
Mean furrow stream			
concentration Treatment (T)	***	0.44	***
Cumulative furrow nutrient			
losses Treatment (T)	***	0.37	***

* $P \leq 0.05$

** $P \leq 0.01$.

influenced dissolved $\text{NH}_4\text{-N}$ concentrations in runoff water samples, but not the computed mean furrow stream concentrations or nutrient mass losses.

Water Samples

Sample Filtration in the Field. When filtered immediately after collection, water sample $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and DRP concentrations were stable regardless of treatment. Thus nutrient concentrations were not affected by extended storage or boric acid addition if samples were filtered in the field (Figures 1a–1c).

Sample Filtration Delayed 10 Days

Delaying sample filtering by 10 days followed immediately by analysis produced measured $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and DRP sample concentrations similar to those from field-filtered samples. This was true whether or not boric acid was added (Figures 1a–1c).

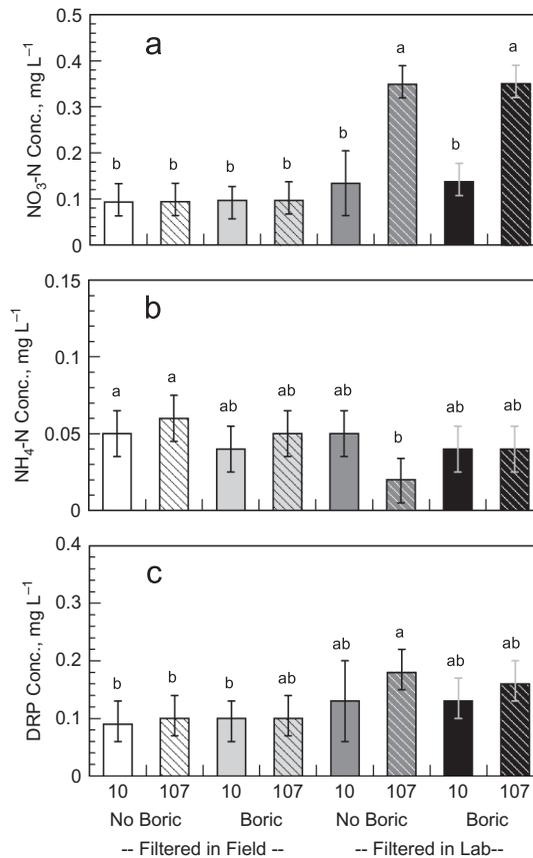


Figure 1. Effects of delayed filtration and storage (10 day vs. 107 day) with or without boric acid biocide on dissolved $\text{NO}_3\text{-N}$ (a), $\text{NH}_4\text{-N}$ (b), and reactive phosphorus (c) concentrations measured in runoff water samples. Error bars represent 95% confidence limits on the means ($n=28$).

Sample Filtration Delayed 10 Days and Stored 107 Days until Analysis

The 10-day delay in filtering the collected sample coupled with a nearly 3-month delay in the analysis caused measured $\text{NO}_3\text{-N}$ concentrations in water samples to increase 3.7-fold beyond that of the field-filtered samples (Figure 1a). The addition of boric acid did not prevent the increase in sample $\text{NO}_3\text{-N}$ concentration during the extended storage. Delayed filtration and extended storage reduced measured $\text{NH}_4\text{-N}$ concentrations in runoff water samples 68% compared to the field-filtered, extended-storage treatment (Figure 1b). The decline in $\text{NH}_4\text{-N}$ concentration for delayed-filtration, extended-storage samples was eliminated when samples were treated with boric acid. The delayed filtration and extended storage treatment increased measured DRP concentrations 1.8-fold relative to field-filtered counterparts (Figure 1c). However, this difference in DRP concentrations was smaller and no longer significant when the delayed-filtration, extended-storage, treated water sample was also treated with boric acid.

Effect of Sediment. Sediment concentration in irrigation inflow water was $\leq 0.1 \text{ g L}^{-1}$, whereas sediment in furrow-stream water samples ranged from 3.1 to 30.8 g L^{-1} . Sediment potentially is a source of (1) soluble inorganic nutrients, which are leached or desorbed from mineral and organic solids, (2) dissolved and particulate organic materials, and (3) bacteria, viruses, and protozoa. Filtering (0.45 μm) removes the solids and most of the bacteria and protozoa from the water sample but leaves viruses, mineral and organic colloids, dissolved organic carbon, and a reduced number of bacteria (Robards et al. 1994). The immediate removal of sediment in the field-filtered, runoff water samples stabilized dissolved nutrient concentrations during extended storage. Permitting runoff sediment to remain in the collected sample for 10 days prior to analysis had little effect on measured nutrient concentrations when compared with the field-filtered samples; however, a trend toward increased $\text{NO}_3\text{-N}$ and DRP concentrations was observed when filtration was delayed (Figures 1a and 1c). This indicates that dominate processes involved were the solubilization or desorption of soluble nutrients associated with sediment and/or mineralization of organic N compounds (Klingaman and Nelson 1976; Bjorneberg et al. 2006).

After extended storage of the delayed filtration samples, however, the earlier transitory presence of sediment in the collected samples resulted in greater measured $\text{NO}_3\text{-N}$ (Figure 1a) and DRP (for no boric acid) concentrations (Figure 1c) and decreased $\text{NH}_4\text{-N}$ concentrations (for no boric acid) (Figure 1b) relative the field-filtered samples. For $\text{NO}_3\text{-N}$ and DRP concentrations in delayed filtration samples, the increase in nutrient concentration caused by extended storage was positively correlated with sample sediment concentration (Table 2). This suggests that soluble or colloidal components associated with the sediment were responsible for nutrient changes during extended storage. The cause of the $\text{NO}_3\text{-N}$ increase during extended storage is difficult to explain abiotically. Because filtering likely did not remove all microorganisms in the water sample, the increase in $\text{NO}_3\text{-N}$ suggests that colloidal and dissolved organic matter and organic N was mineralized (Klingaman and Nelson 1976; Brookshire et al. 2005). The lack of boric acid effects on the $\text{NO}_3\text{-N}$ increase, however, argues against a biotic process being involved. By contrast, boric acid did reduce the effect of extended storage on measured $\text{NH}_4\text{-N}$ and DRP concentrations, suggesting that nitrification caused the observed decrease in $\text{NH}_4\text{-N}$ and mineralization caused the increase in DRP during this period.

Table 2

Pearson's correlation coefficients and *P* values describing the relationship between water sample sediment concentration and the difference between early and late measured nutrient concentration values (day 107 minus day 10) for each treatment

	NO ₃ -N	NH ₄ -N	DRP
Field filter, no boric	-0.26	-0.04	-0.38*
Field filter + boric	0.07	-0.22	-0.06
Delayed filt., no boric	0.60***	-0.30	0.60***
Delayed filt. + boric	0.65***	-0.12	0.60***

**P* ≤ 0.05.

***P* ≤ 0.01.

Computed Furrow Stream Parameters

Sample filtering and storage management effects on measured nutrient concentrations produced corresponding effects on irrigation furrow stream parameters (Table 1). The average furrow stream NO₃-N concentrations and mass losses determined using delayed-filtration, extended-storage samples (with or without boric acid) were significantly different from, and 4.7 times greater than, the average values determined using the other sample treatments (Table 3). Similarly, the mean furrow stream DRP concentrations and mass losses determined using delayed-filtration, extended-storage samples (with or without boric acid) were significantly different from, and nearly 1.9 times greater than, the average values determined using the field-filtered and delayed filtration (no boric acid) treatments (Table 3). Sample treatments had no effect on computed furrow stream concentrations and mass losses of NH₄-N (Table 1).

Both the field-filtered (10- or 107-day storage) and delayed-filtration (10-day storage without boric acid) water samples produced stable, comparatively similar irrigation furrow stream concentration and mass loss values for inorganic dissolved nutrients. Because of their stability, these values can more accurately measure the effects of field practices on runoff nutrient losses at the edge of the field. However, the current research shows that even the transitory presence of sediment in runoff water can alter the water's dissolved nutrient concentrations with time. One may reasonably assume that a volume of furrow runoff water remains associated with its sediment for a time after it enters tail-water ditches, and the water volume may transit through the return flow channels, and eventually the natural surface water system, for extended periods. Because this scenario is not unlike that of the delayed-filtration, extended-storage treatment used in this study, one may infer that dissolved nutrient loads in downstream water bodies will differ substantially from nutrient concentrations measured in runoff at the field edge. This is due to coupled cycling of dissolved organic N, P, and C in the stream and adsorption/desorption processes, which alter dissolved inorganic N and P concentrations as water flows downstream (Brookshire et al. 2005; Bjorneberg et al. 2006). Thus it is unlikely that field edge measurements can be used to reliably predict the potential effect of field management practices on dissolved nutrient concentrations in downstream water bodies. This study suggests that an incubation test of field-edge runoff water may provide a more useful estimate of resulting downstream watershed effects.

Table 3
Sample treatment and storage-time effects on calculated irrigation furrow values for runoff nutrient concentrations and cumulative nutrient losses

Parameter	Nutrient	Field filtered (0 days)						Lab Filtered (10 days)					
		No boric acid		Boric acid		No boric acid		Boric acid		No boric acid		Boric acid	
		10 days ^a	107 days	10 days	107 days	10 days	107 days	10 days	107 days	10 days	107 days	10 days	107 days
Mean runoff concentration (mg kg ⁻¹)	NO ₃ -N	0.07b ^b	0.06b	0.06b	0.06b	0.06b	0.08b	0.35a	0.11b	0.36a			
	NH ₄ -N	0.05	0.04	0.03	0.03	0.03	0.05	0.03	0.03	0.03			
	DRP	0.11b	0.11b	0.11b	0.11b	0.11b	0.14ab	0.22a	0.17ab	0.22a			
Calculated runoff losses (kg ha ⁻¹)	NO ₃ -N	18.4b	16.3b	17.1b	16.7b	21.5b	29.0b	92.2a	29.0b	95.4a			
	NH ₄ -N	12.9	12.5	6.6	8.3	14.8	8.7	8.6	8.7	8.1			
	DRP	28.5c	28.3c	28.6c	28.6c	37.4c	45.7b	57.8a	45.7b	58.3a			

^aStorage days.

^bFor a given parameter and nutrient, treatment means with the same lowercase letter are not significantly different ($P \leq 0.05$). Letters are not displayed if effect was not significant in the ANOVA

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