

## Using Nitrogen-15 to Quantify Vegetative Buffer Effectiveness for Sequestering Nitrogen in Runoff

A. Bedard-Haughn,\* K. W. Tate, and C. van Kessel

### ABSTRACT

Previous studies have observed higher levels of soluble nutrients leaving vegetative buffers than entering them, suggesting that the buffers themselves are acting as a source rather than a sink by releasing previously stored nutrients. This study used 98 atom %  $^{15}\text{N}$ -labeled  $\text{KNO}_3$  at a rate of  $5 \text{ kg ha}^{-1}$  to quantify buffer efficiency for sequestering new inputs of  $\text{NO}_3^-$ -N in an extensively grazed irrigated pasture system. Buffer treatments consisted of an 8-m buffer, a 16-m buffer, and a nonbuffered control. Regardless of the form of runoff N ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , or dissolved organic nitrogen [DON]), more  $^{15}\text{N}$  was lost from the nonbuffered treatments than from the buffered treatments. The majority of the N attenuation was by vegetative uptake. Over the course of the study, the 8-m buffer decreased  $\text{NO}_3^-$ - $^{15}\text{N}$  load by 28% and the 16-m buffer decreased load by 42%. For  $\text{NH}_4^+$ - $^{15}\text{N}$ , the decrease was 34 and 48%, and for  $\text{DON}$ - $^{15}\text{N}$ , the decrease was 21 and 9%. Although the buffers were effective overall, the majority of the buffer impact occurred in the first four weeks after  $^{15}\text{N}$  application, with the buffered plots attenuating nearly twice as much  $^{15}\text{N}$  as the nonbuffered plots. For the remainder of the study, buffer effect was not as marked; there was a steady release of  $^{15}\text{N}$ , particularly  $\text{NO}_3^-$  and  $\text{DON}$ - $^{15}\text{N}$ , from the buffers into the runoff. This suggests that for buffers to be sustainable for N sequestration there is a need to manage buffer vegetation to maximize N demand and retention.

**B**UFFERS ARE STRIPS OF VEGETATION adjacent to agroforestry or agricultural production that function to remove pollutants by reducing or filtering surface runoff and/or by filtering ground water and stream water (Dosskey, 2001). The relative importance of different buffer functions varies according to buffer characteristics such as hydrology, vegetation type (grass vs. forest), soil type (coarse vs. fine), buffer width, and pollutant type (Bharati et al., 2002; Schmitt et al., 1999). Installing buffers without sufficient consideration of these characteristics may result in a tendency to overestimate the effectiveness of buffers (Dosskey, 2002).

There has been limited research on buffer efficiency and capacity in an extensively grazed irrigated pasture system. In California, irrigated pasture provides a relatively low-cost source of green forage during the summer months when surrounding rangelands are dry and dormant. Irrigation rates vary by irrigation method, but for flood irrigation are as high as  $70 \text{ L s}^{-1}$  at the top of the slope, applied continuously over an 8- to 14-h period. In the Sierra Nevada foothills, with slopes from 5 to 30%, this can generate runoff losses of up to 70% (Tate et al., 2000b). Given that irrigated pasture is both fertilized and grazed, there is concern that runoff water con-

tains dangerous levels of pathogens and nutrients. This study is part of a larger project examining buffer effectiveness in irrigated pasture for attenuating N, P, and C, as well as indicator bacteria fecal coliforms and *Escherichia coli*. The component emphasized here is  $\text{NO}_3^-$ , a soluble nutrient commonly implicated in eutrophication in seawater and fresh water (Cole et al., 2004);  $\text{NO}_3^-$  concentrations as low as  $1 \text{ mg L}^{-1}$  can contribute to algal blooms (Mendez et al., 1999).

Nitrate removal is typically attributed to denitrification, infiltration, or plant uptake. Denitrification, particularly in saturated riparian zones, is frequently viewed as the most effective way to prevent  $\text{NO}_3^-$  contamination of surface and ground water (Casey et al., 2001; Hill, 1996). This presents two concerns. First, denitrification rates vary both spatially and temporally, creating predictive challenges (Hill, 1996). For example, Lowrance et al. (1995) observed much higher denitrification rates in grassed areas compared to either hardwood or pine forest buffers. They also observed significant temporal differences related to timing of N application. In addition, buffer design for  $\text{NO}_3^-$  removal via denitrification can only be effective when site-specific hydraulic characteristics are taken into consideration (Aravena et al., 2002; Leeds-Harrison et al., 1999; Sabater et al., 2003). For example, Wigington et al. (2003) found that even high denitrification potential did not guarantee high levels of  $\text{NO}_3^-$  removal because only a small percentage of the stream flow at their study site intersected riparian soils; the majority of the flow came from ephemeral swales. The second concern is that in landscapes receiving high  $\text{NO}_3^-$  inputs, and where denitrification is dominant, riparian buffer zones can serve as significant sources for  $\text{N}_2\text{O}$ , a greenhouse gas with a warming potential 300 times that of  $\text{CO}_2$  (Groffman et al., 1998, 2000; Hefting et al., 2003). In irrigated pasture, hydrologic patterns and associated denitrification potential can be difficult to characterize because they can change drastically with the rapid wet-dry cycles corresponding to irrigation events. Thus, it becomes important to consider the potential for removing  $\text{NO}_3^-$  via infiltration and uptake as opposed to denitrification. As noted by Verchot et al. (1997), infiltration and vegetative uptake can be the dominant factors for attenuating nutrients in surface runoff.

Previous estimates of buffer  $\text{NO}_3^-$  attenuation range broadly, from buffers serving as a net source of  $\text{NO}_3^-$  to buffering effectiveness of >99% (Dillaha et al., 1989; Dosskey, 2001; Hill, 1996). A similarly broad range of 10 to 90% has been observed for  $\text{NH}_4^+$  (Dillaha et al., 1989; Dosskey, 2001). Although there is very little data available on DON in surface runoff, Dosskey's (2001)

Department of Agronomy and Range Science, University of California, Davis, CA 95616. Received 18 Feb. 2004. \*Corresponding author (bedardhaughn@ucdavis.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

**Abbreviations:** DON, dissolved organic nitrogen.

review on buffer effectiveness indicates that for total N, buffers can be either a net sink (up to 91% reduction) or a net source, with up to 50% more total N flowing out of the buffer than into it. This broad range of effectiveness values may be attributable in part to the mechanism for N removal. With denitrification,  $\text{NO}_3^-$  is removed from the terrestrial and aquatic systems; in contrast, infiltration and uptake may provide only ephemeral storage. Dillaha et al. (1989) attributed high levels of soluble nutrients leaving buffers to low trapping efficiency for soluble nutrients and release of nutrients previously trapped in the filter. Buffer trapping efficiency may decrease over time, and buffers may ultimately become a source of N rather than a sink (Mendez et al., 1999). The role of N cycling within the buffers must not be neglected when considering potential sources of N,  $\text{NO}_3^-$  or otherwise.

Stable  $^{15}\text{N}$  isotopes are used to study the fate and transport of N. Previous buffer studies using  $^{15}\text{N}$  have focused on natural abundance methods, using naturally occurring variations in  $^{15}\text{N}$  levels to identify pollutant sources that were moving through buffers to adjacent waterways (Chang et al., 2002; Karr et al., 2003; Spruill et al., 2002), or to determine whether or not denitrification was a major factor in  $\text{NO}_3^-$  removal (Dhondt et al., 2002; Ostrom et al., 2002). However,  $^{15}\text{N}$  natural abundance provides, at best, semiquantitative estimates of pathways and processes occurring in the field (Bedard-Haughn et al., 2003). If not completely accounted for, background variability in isotopic signatures and fractionating processes that alter those signatures to varying levels can confound interpretation of  $^{15}\text{N}$  data. Even when sources of variability are accounted for, natural abundance techniques do not allow differentiation between new sources of N and N already stored within the system. In contrast, using  $^{15}\text{N}$ -enriched isotopes allows new N sources to be quantitatively traced through the system and measured in the various potential sinks, and the  $^{15}\text{N}$  level of the applied tracer can be predetermined to ensure that the signature is detectable above background variability, even when fractionation occurs (Bedard-Haughn et al., 2003). Isotopic levels are reported as the amount of  $^{15}\text{N}$  present relative to the average naturally occurring background  $^{15}\text{N}$  levels for a given source. There have been a limited number of studies using  $^{15}\text{N}$ -enriched tracers in the field (Davidson et al., 1990; Di et al., 1999; Mulholland et al., 2000), due primarily to high tracer cost. We were unable to find any previous field studies that used  $^{15}\text{N}$ -enriched tracers to quantify buffer effectiveness for attenuating  $\text{NO}_3^-$ . Previous work by Matheson et al. (2002) to quantify the fate of  $^{15}\text{N}$  tracers in riparian zones was performed under controlled laboratory settings as microcosm studies. They determined that soil immobilization and plant assimilation accounted for less than half of the applied tracer; the remainder (61–63%) was assumed to have been lost via denitrification. They could not, however, account for any lateral or vertical movement that might occur in a natural field setting.

Given previous evidence suggesting that vegetative buffers themselves are acting as a pollutant source

rather than a sink by releasing previously stored nutrients, the major objectives of this study were to determine (i) if buffers in irrigated pasture were effective in sequestering new sources of  $\text{NO}_3^-$ , (ii) where sequestered  $\text{NO}_3^-$  was being stored, and (iii) whether the added  $\text{NO}_3^-$  remained sequestered in the buffers or was subsequently lost, either as  $\text{NO}_3^-$  or as a different form of N (i.e., buffer sustainability). The data were examined both for overall effectiveness in sequestering N over the course of the summer and for general trends in N uptake.

## MATERIALS AND METHODS

### Site Description

The University of California Sierra Foothill Research and Extension Center (SFREC), located 100 km northeast of Sacramento, California, has a xeric climate and hilly terrain. During the summers of 2000 and 2001, nine adjacent plots were established within an existing flood-irrigated pasture at the SFREC (Fig. 1). A completely random study design was employed to allocate three buffer treatments in three replicates to nine plots. Buffer treatments consisted of a 3:1 pasture to buffer area ratio, a 6:1 pasture to buffer area ratio, and a no-buffer control. Each plot had a 240-m<sup>2</sup> (5 m wide by 48 m long) pasture area. The 3:1 pasture to buffer area treatment had a buffer area of 80 m<sup>2</sup>, and the 6:1 pasture to buffer area treatment had a buffer area of 40 m<sup>2</sup>. Buffer length for the 3:1 and 6:1 buffer treatment was 16 and 8 m, respectively. Plots were established parallel to the slope and the direction of irrigation flow (Fig. 1).

The pasture–buffer areas were dominated by orchardgrass (*Dactylis glomerata* L.), Yorkshire fog/velvetgrass (*Holcus lanatus* L.), and dallis grass (*Paspalum dilatatum* Poir.), with purpletop/tall verbena (*Verbena bonariensis* L.) also present in the buffer areas. Soils (Table 1) were classified as fine-loamy, mixed, thermic, Mollic Haploxeralfs of the Auburn–Las Posas–Argonaut rocky loam association (Herbert and Begg, 1969). Slope ranged from 9.5 to 11.9%. The pasture area was fertilized with 170 kg ha<sup>-1</sup> of 16–20–0 (N–P–K) in early May. Grazing in pasture areas was by mature beef cattle at a stocking density of 5 animal units (dry cow) on 0.216 ha for 2 d. Cattle were managed to replicate grazing and fecal loading rates typical of the region. Mean fecal loading rate per grazing event was 336 kg ha<sup>-1</sup> plot<sup>-1</sup> ( $\pm 29.1$ ). A 3-wk rest period was maintained between grazing events to assure the sustained health and productivity of the pasture's vegetation. Buffer areas were neither fertilized nor grazed, but received the same irrigation treatment as the pasture areas.

Irrigation water was applied every 11 d from April through October via adjustable flow rate or “gated” irrigation pipe. During this project, the irrigation rate was calibrated to 4 L s<sup>-1</sup> per treatment for approximately 3.5 h (167 L s<sup>-1</sup> ha<sup>-1</sup>). These rates were typical of flood-irrigated pasture in this region; pasture areas were managed to minimize the occurrence of channelized flow. Earthen berms separated adjacent areas to prevent water crossing from one treatment to another. Polyvinyl chloride collection troughs, with a V-notch at one end for sample collection, were installed across the bottom of each treatment with the edge of the trough flush with the ground surface. Concrete was used to prevent erosion along the edge of the troughs. Troughs collected all surface water runoff, allowing for the measurement of surface water runoff rates and collection of water samples for analysis. Collection troughs were fenced to exclude cattle. Subsurface water was

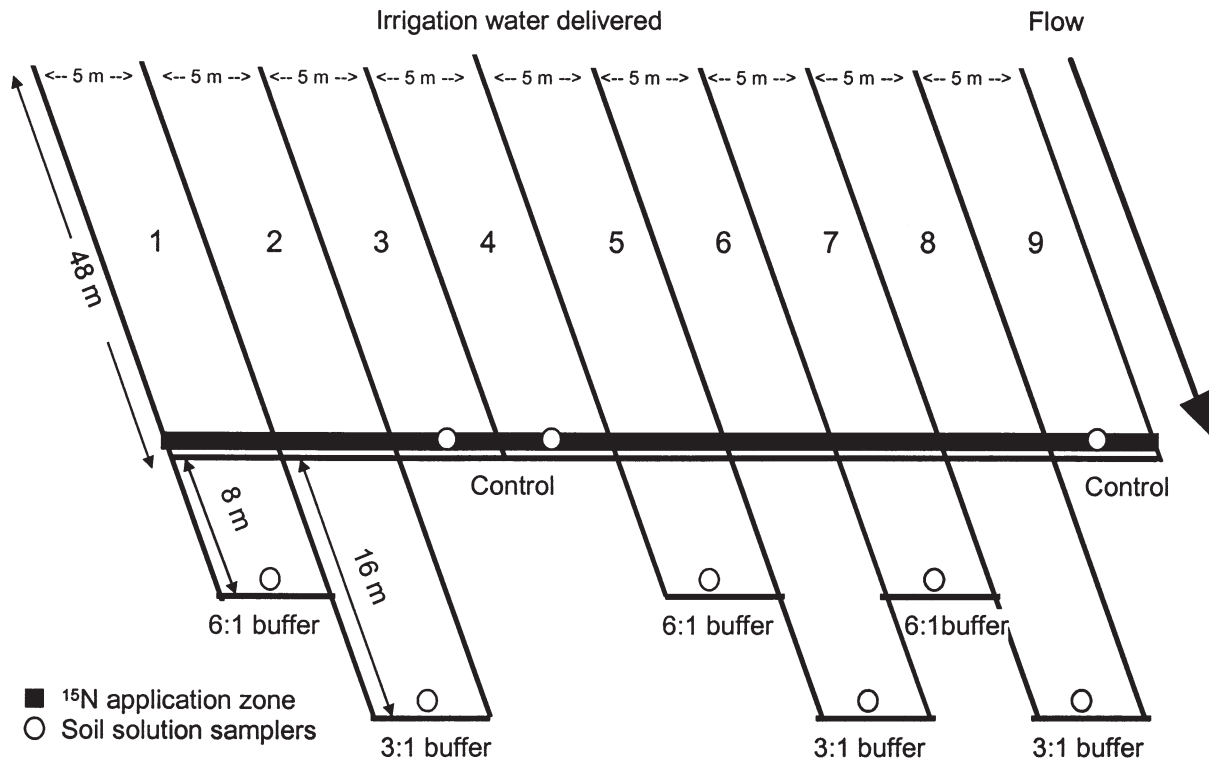


Fig. 1. Schematic of plot design (not to scale). Collection troughs installed at the bottom of each treatment (downslope of solution samplers).

collected using soil solution samplers (Soilmoisture Equipment, Santa Barbara, CA), which were installed to a depth of 45 cm, the approximate depth of the heavy clay Bt horizon (Fig. 1).

### Nitrogen-15 Application and Analysis

Nitrogen isotopes, which are stable and nonradioactive, have been used extensively to follow the dynamics of N in soils and crops (Powlson and Barraclough, 1993). We used  $^{15}\text{N}$  enriched material so that the added N could be detected and differentiated from inherent background variability in naturally occurring  $^{15}\text{N}$  levels (Bedard-Haughn et al., 2003). Natural abundance background levels of  $^{15}\text{N}$  in all N pools were measured before application of  $^{15}\text{N}$ -labeled fertilizer to account for natural variability and dilution of the applied  $^{15}\text{N}$  fertilizer by background  $^{14}\text{N}$ .

In July 2002,  $^{15}\text{N}$ -labeled  $\text{KNO}_3$  was applied in solution at a rate of  $5 \text{ kg N ha}^{-1}$  and 99.7 atom %  $^{15}\text{N}$ . The rate and atom % concentration were selected to provide an approximation of post-irrigation fertilizer N levels while allowing the tracer to be detectable in all N pools throughout the duration of the experiment. The  $^{15}\text{N}$  solution was applied across all nine plots along the entire width of the experiment. The area labeled

was 1 by 5 m wide and located 0.75 m above the buffer areas. Following application, the labeled fertilizer was watered in with  $20 \text{ L}$  of water  $\text{m}^{-2}$ . Watering in was done by hand with watering cans for maximum precision;  $20 \text{ L}$  represented the optimum amount to ensure that the applied  $^{15}\text{N}$ -labeled  $\text{KNO}_3$  was rinsed off of the foliar surfaces, but the volume was not so great as to cause deep leaching of the applied fertilizer. The  $^{15}\text{N}$  application area was fenced to prevent redistribution of the  $^{15}\text{N}$ -enriched material by the cattle.

For a 14-wk period following application, water samples were collected from the installed collection troughs during each irrigation trial (11-d schedule). Water samples ( $500 \text{ mL}$ ) were collected as "grab" samples from the V-notch at the end of each collection trough. Samples were taken at 0 (leading edge of runoff), 15, 30, 60, 90, and 120 min following commencement of runoff from each treatment and were stored frozen until analysis. This sampling scheme represented a minimum sample number and is based on previous experience with the timing of runoff and pollutant transport from these systems. At each sampling interval, runoff rate was determined by measuring the volume of runoff draining from the V-notch in the collection trough in a 5-s period. Runoff rate data were used to determine runoff losses (Table 1). Following each irrigation, vacuum was applied to the soil solution sampling tubes and allowed to draw moisture from the soil for 10 d (i.e., until the next irrigation). Although vacuum was not applied constantly over the 10-d period, suction was still present at sampling. Soil water samples were collected just before the subsequent irrigation and were stored frozen until analysis.

Runoff  $^{15}\text{N}$  isotope analyses were performed on all three N pools:  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and total N for Days 1, 12, 31, 65, and 86 following application of the tracer. For Days 1 and 12, only the 0-, 15-, 60-, and 120-min intervals were analyzed because preliminary experiments indicated that this was sufficient for characterization of maximum variation. For Days 31 to 86, even fewer intervals were needed to acquire sufficient infor-

Table 1. Field site properties averaged across all treatments.

Property	Value (mean $\pm$ SD)
C, %	3.0 $\pm$ 0.4
N, %	0.3 $\pm$ 0.04
C to N ratio	10.4 $\pm$ 0.4
Sand, %	30.0 $\pm$ 3.6
Silt, %	33.8 $\pm$ 1.1
Clay, %	36.2 $\pm$ 2.9
Slope, %	10.9 $\pm$ 0.8
Runoff losses, % <sup>†</sup>	56.8 $\pm$ 16.4

<sup>†</sup> Runoff volume/irrigation volume; averaged over multiple irrigation events.

mation because there was no longer significant change between sampling days. Samples were filtered to remove sediment and vegetation residues from runoff. Ammonium  $^{15}\text{N}$  and  $\text{NO}_3^-$ - $^{15}\text{N}$  were determined by  $\text{NH}_3$  diffusion onto polytetrafluoroethylene-encased acid traps (Stark and Hart, 1996). To measure  $\text{NO}_3^-$ - $^{15}\text{N}$ , the Stark and Hart (1996) method was modified only slightly in that following diffusion of 100-mL samples for  $\text{NH}_4^+$ , 1 mL of 5 M NaOH was added to each to bring the pH up to  $\geq 12$ . Samples were heated uncovered at 95°C to remove any trace ammonium or labile organic N (DON) and to concentrate the volume down to 25 mL. In place of Devarda's alloy,  $\text{TiCl}_3$  (Fisherbrand Titanous Chloride Solution, 20%; Fisher Scientific, Hampton, NH) was then added (typically one-twentieth of the sample volume) to reduce  $\text{NO}_3^-$  to  $\text{NH}_3$ . Soil solution samples (25-mL aliquots) were analyzed for  $\text{NO}_3^-$ - $^{15}\text{N}$  via the  $\text{TiCl}_3$  diffusion as above, except no concentrating step was required. Titanous chloride has been found preferable to Devarda's alloy due to its low cost, low N contamination, and availability in solution form (Cho et al., 2002; Cresser, 1977; Crumpton et al., 1987). Samples were sealed and incubated at 50°C for 72 h. Nitrate standards with field-level N concentrations had mean N recovery of 94% (SD  $\pm$  5%) using this modified method.

Total  $^{15}\text{N}$  was determined on a separate 20-mL aliquot by performing a persulfate digestion (American Public Health Association, 1989) to convert the DON and  $\text{NH}_4^+$  to  $\text{NO}_3^-$ , and samples were then diffused for  $\text{NO}_3^-$  as above (without concentration step). The DON- $^{15}\text{N}$  for each sample was calculated using an isotope mixing model via difference from total  $^{15}\text{N}$  (Shearer and Kohl, 1993):

$$^{15}\text{N}_{\text{DON}} = \frac{^{15}\text{N}_{\text{NT}}m_{\text{NT}} - ^{15}\text{N}_{\text{NH}_4}m_{\text{NH}_4} - ^{15}\text{N}_{\text{NO}_3}m_{\text{NO}_3}}{m_{\text{NT}}} \quad [1]$$

where  $^{15}\text{N}_x$  refers to the atom % value for a given N form and  $m_x$  refers to the quantity of N in  $\mu\text{g}$ .

Following diffusion, acid disks were removed from polytetrafluoroethylene packets and analyzed via mass spectrometry (Integrated Stable Isotope Analyzer; Europa Integra, Crewe, UK) at the University of California-Davis Stable Isotope Facility. The current sensitivity of our stable isotope ratio mass spectrometers is 0.0002 atom %  $^{15}\text{N}$ .

Representative plant samples from the pasture and buffer areas were taken before each irrigation trial. To determine how far the  $^{15}\text{N}$  fertilizer had moved into the buffer strip, plants were sampled across the width of the buffer at down slope intervals with a sample spacing of 1 m immediately above and below the zone of  $^{15}\text{N}$  application, and spacing of 2 m further into the buffer. The buffer vegetation samples were separated between grasses and verbena, the native shrub in the buffers. Following each grazing (every second irrigation), the fenced  $^{15}\text{N}$  application area was clipped and the vegetation removed to simulate grazing. All plant samples were oven-dried at 65°C and analyzed for  $^{15}\text{N}$  isotopic composition via mass spectrometry (van Kessel et al., 1994).

Soil samples were taken monthly to a 15-cm depth in two increments (0–7 and 7–15), corresponding to the depth of the A horizon. Samples were taken at 0, 1, and 5 m from the  $^{15}\text{N}$  application zone at 12, 43, and 86 d following  $^{15}\text{N}$  application. Samples were also taken at 8 and 16 m on Day 86. Sample quantity, depth, and diameter were limited due to concurrent sampling at the site to analyze total suspended sediment in runoff. Soil samples were oven-dried at 40°C and analyzed for total N and  $^{15}\text{N}$  via mass spectrometry.

Isotopic levels for the soils and plants are reported as atom %  $^{15}\text{N}$  excess, which refers to the amount of  $^{15}\text{N}$  present relative to the average naturally occurring background  $^{15}\text{N}$

levels for that particular source. Background levels are based on pre-application samples. Where possible, atom %  $^{15}\text{N}$  excess amounts were extrapolated to get the total amount of  $^{15}\text{N}$  in a given pool by weight and thus to determine a  $^{15}\text{N}$  budget. Note that it was not possible to perform budget calculations for the vegetation in the buffer areas as accurate biomass measurements over the course of the summer season would have required destructive sampling that would have confounded subsequent measurements.

### Statistical Analysis

The results were analyzed using linear mixed effects model analysis. Linear mixed effects analysis can be applied to both structured and observational studies (Pinheiro and Bates, 2000) and was used here to account for the influence of both fixed (buffer treatment) and random (irrigation date) effects on buffer  $^{15}\text{N}$  uptake levels. Treating time as a random effect provided a direct test for whether buffered plots were significantly different from nonbuffered plots when results were considered over the duration of the study. The magnitude and direction ( $\pm$ ) of the coefficient for buffer effect was used to define the relationship between  $^{15}\text{N}$  loading in runoff and buffer treatment. This approach allowed for robust evaluation of the data while accounting for the repeated measures (group effect–plot identity) embedded in the data structure. This flexible model also allowed within-group variance and correlation structures for handling within-group (plot) heteroscedasticity and temporally correlated errors (irrigation series within year) (Pinheiro and Bates, 2000). This approach has been used in modeling other complex longitudinal datasets (Atwill et al., 2002; Tate et al., 2000a, 2003).

## RESULTS

With few exceptions, the nonbuffered treatment had the highest runoff concentrations of  $^{15}\text{N}$ , with the difference between the buffered and nonbuffered treatments being greatest at the leading edge of runoff ( $t = 0$ ) and diminishing over the course of a given irrigation event (Fig. 2). Following the leading edge, the concentration increased slightly for the  $\text{NO}_3^-$ - and DON- $^{15}\text{N}$  pools, and then decreased corresponding to a rapid increase in runoff levels as the irrigation proceeded. Typically, initial ( $t = 0$ ) runoff levels were approximately 0.4 L  $\text{s}^{-1}$  plot $^{-1}$ , increased rapidly to 2 L  $\text{s}^{-1}$  plot $^{-1}$  by 30 min, and then leveled at a steady rate of approximately 3 L  $\text{s}^{-1}$  plot $^{-1}$  by 60 min. During the second post-application irrigation (Day 12), the  $\text{NO}_3^-$ - $^{15}\text{N}$  concentration started similar to the concentration at the end of the previous irrigation, but for the other pools, there was a slight increase in concentration at the leading edge of runoff. By Day 31, the pattern was well established, with a slight increase in concentration at the start of each irrigation event, followed by a rapid decrease to a steady level. The  $\text{NO}_3^-$ - $^{15}\text{N}$  levels showed the greatest change over the course of the summer, from having the highest concentration at Day 1 to the lowest at Day 86. The  $\text{NH}_4^+$ - $^{15}\text{N}$  levels tended to remain relatively constant. By Day 31, the DON- $^{15}\text{N}$  pool established a new steady level and remained constant for the remainder of the summer. Differences between the 8- and 16-m buffers could also be observed during some of the earlier irrigations, but did not display the same consistent pattern.

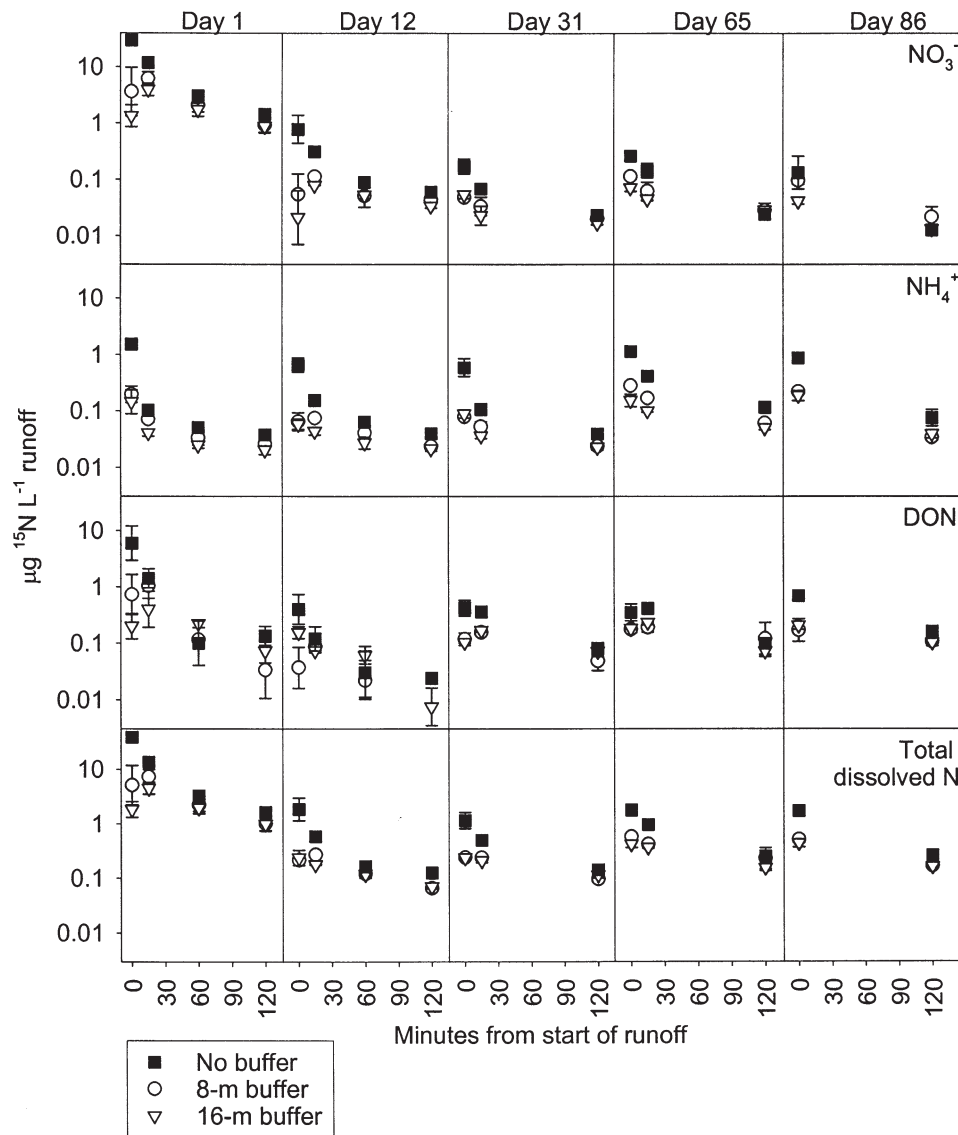


Fig. 2. The  $^{15}\text{N}$  concentrations within and between irrigations. Values are averaged by buffer treatment and time; error bars represent standard errors. Note log y axis.

The total amount of  $^{15}\text{N}$  lost via runoff ( $^{15}\text{N}$  load) during a given irrigation event was determined by multiplying runoff volume by  $^{15}\text{N}$  concentrations for each measured interval and integrating over time (Fig. 3). Regardless of the buffer treatment, maximum  $^{15}\text{N}$  loads were observed in the first irrigation following application. Note, however, that for  $\text{NH}_4^+ - ^{15}\text{N}$ , the loads were relatively low and constant for the first two irrigations following application, and overall, remained quite steady over the course of the summer. Nitrate  $^{15}\text{N}$  load started at a much higher level than the other pools, but decreased rapidly to a lower level and continued to be detectable throughout the summer. Although  $\text{DON} - ^{15}\text{N}$  load decreased after the first irrigation, it established a higher steady-state level, similar to that of  $\text{NH}_4^+ - ^{15}\text{N}$ . Typically, the greatest differences between the buffered and nonbuffered treatments were observed in the first month after  $^{15}\text{N}$  application, but by later in the summer, there were minimal differences among treatments. Note,

however, that by the end of the summer, the buffered treatments occasionally exhibited higher  $^{15}\text{N}$  loads for  $\text{NO}_3^-$  and  $\text{DON}$  than the nonbuffered treatment (Fig. 3).

Linear mixed effects analysis of the  $^{15}\text{N}$  runoff load over the course of the entire summer indicated that when compared to the nonbuffered treatments, the buffered treatments had significantly less  $^{15}\text{N}$  ( $P = 0.05$ ) for all N pools except for the  $\text{NO}_3^-$  pool in the 8-m buffer and the  $\text{DON}$  pool in the 16-m buffer (Table 2). For the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  pools, the log mean load of  $^{15}\text{N}$  in runoff decreased from the nonbuffered to the 8- to 16-m buffers (from  $e^{-0.19}$  to  $e^{-0.42}$ ), illustrating that  $^{15}\text{N}$  load decreased as buffer length increased (Table 2). In contrast, the log mean load of  $\text{DON} - ^{15}\text{N}$  was greater for the 16- than the 8-m buffer ( $e^{0.06}$  versus  $e^{0.01}$ ), suggesting that although buffered treatments had less  $^{15}\text{N}$  load than nonbuffered, the 8-m buffer had a more substantial effect on load than the 16-m buffer.

There were detectable levels of  $^{15}\text{N}$  in the 45-cm soil

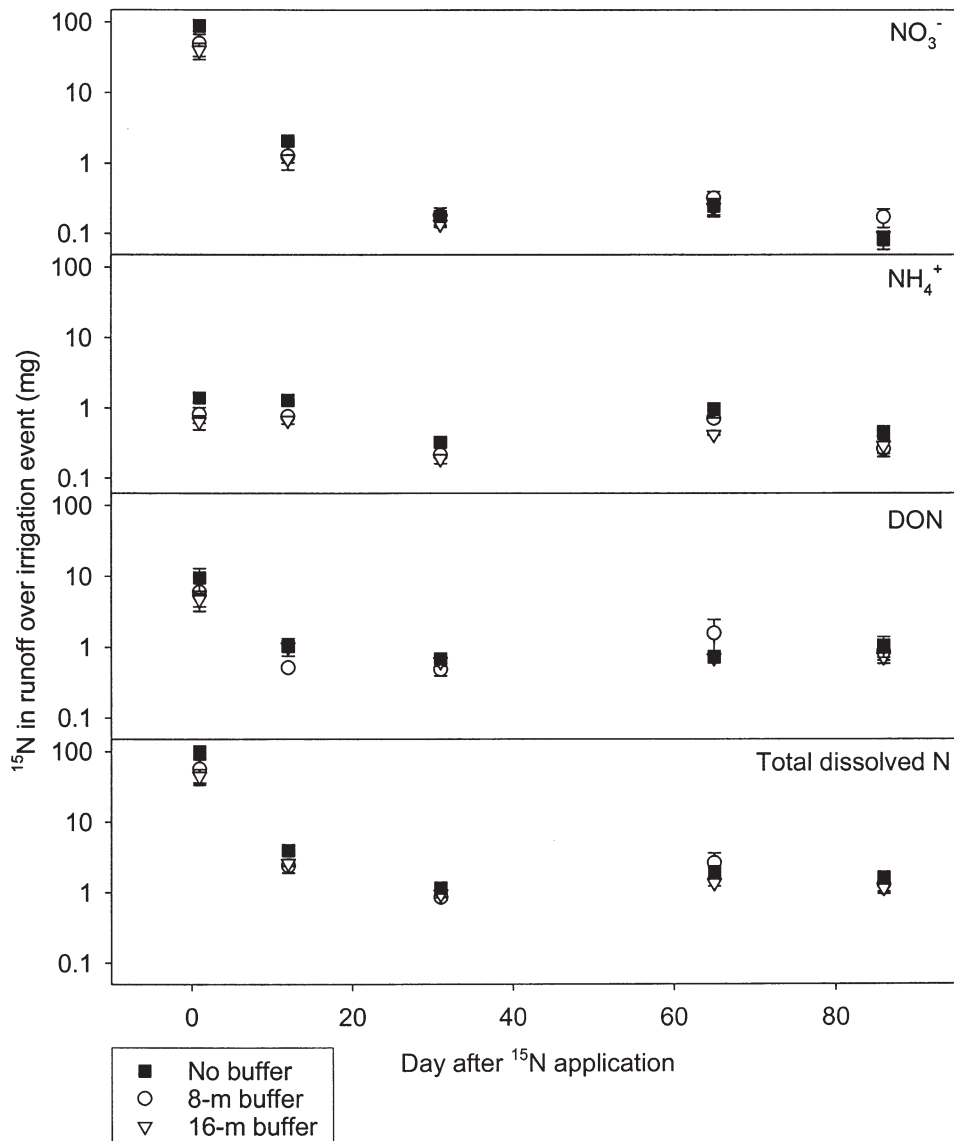
**Table 2. Linear mixed effects analysis of runoff data.**

<sup>15</sup> N pool	Factor	Log mean <sup>15</sup> N load <sup>†</sup> mg (±SD)	Regression coefficient (95% CI) <sup>‡</sup>	P
NO <sub>3</sub> <sup>-</sup>	no buffer	-0.12 ± 2.65	0	
	8-m buffer	-0.19 ± 2.24	-0.33 (-0.86, 0.21)	0.1855
	16-m buffer	-0.42 ± 2.29	-0.56 (-1.09, -0.02)	0.0437
	intercept		1.49 (1.18, 1.81)	<0.0001
NH <sub>4</sub> <sup>+</sup>	no buffer	-0.29 ± 0.63	0	
	8-m buffer	-0.77 ± 0.66	-0.42 (-0.55, -0.29)	0.0002
	16-m buffer	-0.96 ± 0.56	-0.65 (-0.78, -0.52)	<0.0001
	intercept		-0.31 (-0.39, -0.24)	<0.0001
DON <sup>§</sup>	no buffer	0.27 ± 1.04	0	
	8-m buffer	<0.01 ± 1.03	-0.23 (-0.36, -0.10)	0.0046
	16-m buffer	0.06 ± 0.80	-0.10 (-0.23, 0.02)	0.0946
	intercept		-0.40 (-0.48, -0.33)	<0.0001
Total dissolved N	no buffer	1.43 ± 1.70	0	
	8-m buffer	1.12 ± 1.55	-0.45 (-0.52, -0.37)	<0.0001
	16-m buffer	1.01 ± 1.48	-0.33 (-0.41, -0.25)	<0.0001
	intercept		0.73 (0.68, 0.77)	<0.0001

<sup>†</sup> The <sup>15</sup>N load was transformed via natural log to account for greater variability immediately post-application. Negative log mean <sup>15</sup>N values reflect mean values of less than 1 mg (i.e.,  $e^{-0.12} = 0.89$ ,  $e^{0.27} = 1.31$ ).

<sup>‡</sup> Coefficients quantify the expected effect of buffer treatment on log mean <sup>15</sup>N load.

<sup>§</sup> Dissolved organic nitrogen.



**Fig. 3. The <sup>15</sup>N load over the course of the summer. Values are averaged by buffer treatment and time; error bars represent standard errors. Note log y axis.**

**Table 3. Changes in atom % <sup>15</sup>N excess by N pool over the course of the study.**

Days after <sup>15</sup> N	Runoff†	Days after <sup>15</sup> N	<sup>15</sup> N zone vegetation‡	Buffer vegetation‡	Soil solution§	Soil¶
d	atom % <sup>15</sup> N excess (±SD)	d	atom % <sup>15</sup> N excess (±SD)			
1	0.127 ± 0.166	12	3.524 ± 0.684	0.012 ± 0.017	0.018 ± 0.007	0.011 ± 0.018
31	0.008 ± 0.007	43	0.862 ± 0.163	0.007 ± 0.006	0.005 ± 0.002	0.004 ± 0.007
75	NA#	86	0.278 ± 0.076	0.007 ± 0.005	0.005 ± 0.001	0.002 ± 0.004

† Runoff values are for total dissolved N. Background atom % <sup>15</sup>N value = 0.3666.

‡ Vegetation values are for grasses only. Background atom % <sup>15</sup>N value = 0.3659 for <sup>15</sup>N zone vegetation and 0.3667 for buffer vegetation.

§ Soil solution values are for NO<sub>3</sub> only. Background atom % <sup>15</sup>N value = 0.3666.

¶ Soil values are total N. Background atom % <sup>15</sup>N value = 0.3676.

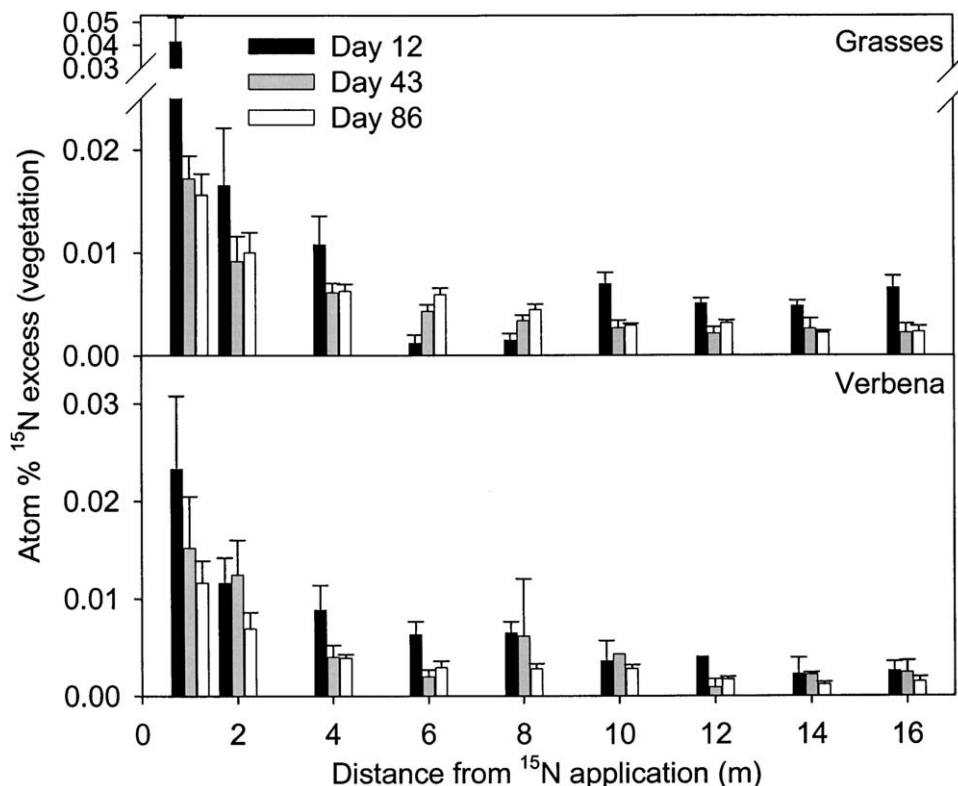
# Runoff <sup>15</sup>N not analyzed for Day 75.

solution samplers (Table 3), with a very slight decrease in atom % <sup>15</sup>N excess from the nonbuffered to the 8-m buffer to the 16-m buffer, but this trend was not statistically significant (data not shown).

The majority of the <sup>15</sup>N not lost via runoff was stored in vegetation and soils. Based on conservative estimates of pasture biomass, approximately 10.3 g (SD ± 1.4) were stored in the pasture grasses immediately underneath the zone of <sup>15</sup>N application within 11 d of application. This represents 46% of the 22.5 g of <sup>15</sup>N applied across all treatments (2.5 g per treatment). By the end of the summer, only 1 g of <sup>15</sup>N (4% of total applied) remained in the pasture biomass, but because the pasture biomass was regularly clipped and removed to simulate grazing, <sup>15</sup>N was actually removed from the system and was not recycled into the buffers. Within the buffers, most of the <sup>15</sup>N was stored in the first 4 m downslope of the zone of application, as indicated by the higher values of atom % <sup>15</sup>N excess (Fig. 4). The amount of <sup>15</sup>N then decreased further downslope, but note that <sup>15</sup>N

was observed in the vegetation at the end of the longest buffer even at the first sampling following application. For the grasses, the <sup>15</sup>N enrichment decreased over time, indicating dilution of the <sup>15</sup>N signature via uptake of non-enriched N. The only exceptions to this dilution occurred at 6 and 8 m downslope. For the verbena, the <sup>15</sup>N enrichment decreased over time for the first 8 m, but generally remained constant further downslope. Between Days 43 and 86, there was very little change in <sup>15</sup>N levels in the vegetation. Additional measurements were performed 3 and 6 mo after the last irrigation (data not shown). Compared to Day 86, there was little change in vegetation <sup>15</sup>N levels at 3 mo, but by 6 mo after the last irrigation, <sup>15</sup>N levels had decreased by approximately 50%.

Of the <sup>15</sup>N applied, approximately 23% was immediately stored in the upper 15 cm of the soil immediately beneath the zone of application (Table 4); however, this was subject to redistribution further downslope during subsequent irrigations (Fig. 5). In the 0- to 7-cm layer,



**Fig. 4. Atom % <sup>15</sup>N excess in vegetation by distance. Values are averaged by time and distance across all treatments; error bars represent standard errors. Data from the <sup>15</sup>N application zone not shown here due to graphical limitations.**

**Table 4. Nitrogen-15 budget for soil and runoff as mean percentage of applied  $^{15}\text{N}$  recovered by buffer treatment.**

Time	Depth cm	Soil % $^{15}\text{N}$ recovery <sup>†</sup>			
		No buffer <sup>‡</sup>	8-m buffer <sup>‡</sup>	16-m buffer <sup>‡</sup>	
Day 12	0-7	17.5 ± 4.3	19.1 ± 6.6	21.7 ± 10.6	
		1.7 ± 0.3	2.5 ± 0.6	6.8 ± 7.7	
	7-15	NA	0.2 ± 0.2	0.3 ± 0.1	
		NA	0.4 ± 0.4	0.6 ± 0.2	
	Day 86	0-7	3.4 ± 3.2	4.6 ± 5.6	2.2 ± 1.7
			0.6 ± 0.6	0.7 ± 0.6	0.3 ± 0.3
7-15		NA	2.2 ± 2.0	2.7 ± 3.1	
		NA	1.2 ± 0.9	1.2 ± 1.2	
Cumulative total (Days 1-86)		Runoff % $^{15}\text{N}$ recovery			
		Form	No buffer	8-m buffer	16-m buffer
	$\text{NH}_4^+$	0.3 ± 0.04	0.2 ± 0.02	0.1 ± 0.01	
	$\text{NO}_3^-$	3.8 ± 1.2	2.1 ± 1.3	1.7 ± 0.7	
DON <sup>§</sup>	0.6 ± 0.2	0.5 ± 0.4	0.4 ± 0.1		
Total dissolved N	4.6 ± 1.4	2.8 ± 1.6	2.2 ± 0.8		

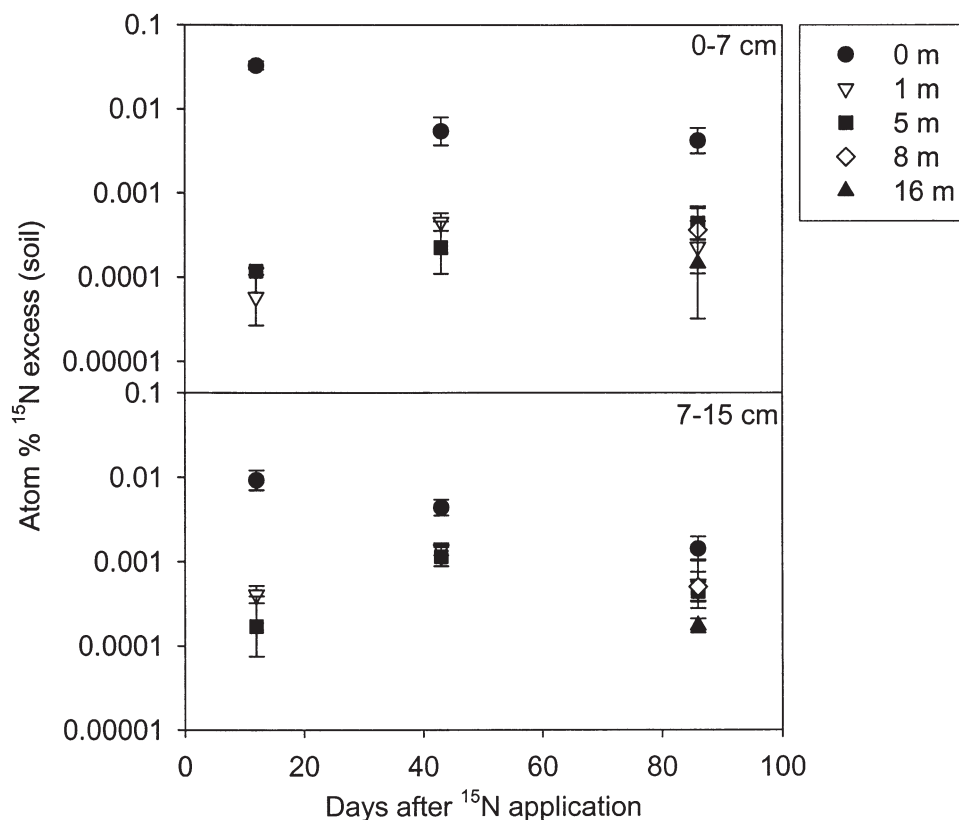
<sup>†</sup> Soil data differentiate between samples taken within the zone of  $^{15}\text{N}$  application and samples taken in the buffer areas. Vegetation values not given due to lack of precise biomass measurements. Differences in soil  $^{15}\text{N}$  between Days 12 and 86 represent losses via runoff, lateral and vertical leaching, denitrification, or volatilization.

<sup>‡</sup> 2500 mg  $^{15}\text{N}$  applied per buffer treatment.

<sup>§</sup> Dissolved organic nitrogen.

the  $^{15}\text{N}$  levels immediately under the zone of application (0 m) decreased over the summer irrigation season. Further downslope, the  $^{15}\text{N}$  levels started lower, and increased over the season, suggesting lateral movement

within the 0- to 7-cm layer. A similar pattern was observed in the 7- to 15-cm layer except that by the end of the season, there was another slight decrease in  $^{15}\text{N}$  levels at all distances. Unlike the vegetation measure-



**Fig. 5. Atom %  $^{15}\text{N}$  excess in soils by time. Values are averaged by time and distance across all treatments; error bars represent standard errors. Eight- and 16-m data only available for Day 86.**



ments, soil measurements 6 mo after the last irrigation indicated similar soil  $^{15}\text{N}$  levels when averaged across all plots, but the spatial distribution changed.

The  $^{15}\text{N}$  tracer was observed in all measured pools (Table 3). Levels were at a maximum for the first sampling date following  $^{15}\text{N}$  application, but within a month of application, levels in all pools had dropped to a lower level of steady enrichment. The  $^{15}\text{N}$  could still be measured within the system but was neither increasing nor decreasing further.

## DISCUSSION

### Buffer Effectiveness

Most of the previous studies on buffer effectiveness fail to differentiate between new N and the fate of N that is already stored in the buffers, and so those results may be either over- or underestimating the effectiveness of buffers.

The  $^{15}\text{N}$  runoff data showed that buffers were effective for sequestering new  $\text{NO}_3^-$  in irrigated pasture over the course of the summer. The regression coefficients in Table 2 demonstrate that for  $\text{NO}_3^-$ , the 8-m buffer decreased  $^{15}\text{N}$  load by approximately 28% and the 16-m buffer decreased load by 42%. Indeed, regardless of the form of N, more  $^{15}\text{N}$  was lost from the nonbuffered irrigated pasture plots than from those with 8- or 16-m buffers. For  $\text{NH}_4^+$ , the decrease was 34% (8 m) and 48% (16 m), whereas for DON, the decrease was 21% (8 m) and 9% (16 m). The net effect on  $^{15}\text{N}$  load, illustrated by the total dissolved N analysis, is a decrease of 36% for the 8-m buffer and 28% for the 16-m buffer, suggesting that DON appears to be the limiting factor in the effectiveness of the buffers, particularly the 16-m buffers.

### Nitrogen Sequestration

In considering vegetative effects, Schmitt et al. (1999) found that although sorghum [*Sorghum bicolor* (L.) Moench] was effective as a vegetative filter, grass buffers had no effect on the concentration of dissolved constituents. In this study, however, the primary mechanism for removal of applied  $^{15}\text{N}$ - $\text{NO}_3^-$  was plant uptake; specifically, grass uptake in the zone of  $^{15}\text{N}$  application. Within 10 d of application, approximately 40 to 50% of the tracer was removed by plant uptake and a further 23 to 27% was stored in soil immediately below the zone of application, accounting for up to 77% of the applied tracer. A further 3% of the applied tracer was observed in the runoff on the first day after application (1.5% from nonbuffered, 0.9% from 8-m buffers, 0.6% from 16-m buffers), resulting in total  $^{15}\text{N}$  recovery of up to 80% just within the pasture and runoff. This is much higher recovery than the 11 to 15% removed by plant uptake and 24 to 26% stored in the soil in the microcosm study by Matheson et al. (2002), which did not account for runoff. The level of plant N uptake in the pasture is less than the 72% measured by Griffith et al. (1997) in a grass-seed production system in western Oregon. However, our irrigated pasture uptake values reflect

only the uptake of added  $^{15}\text{N}$ , not the uptake of N that was already in the system. The overall high levels of plant N uptake observed in irrigated pasture indicate that although there is not a shallow ground water table at this site, the presence of significant fine root biomass associated with the N-P-K-fertilized annual grasses improves the potential for plant uptake (Cheng and Bledsoe, 2002; Hill, 1996).

Further storage and uptake occurred within the buffer, particularly in the first few meters. Although the total amount sequestered in buffer vegetation could not be definitively quantified due to lack of precise biomass measurements, atom %  $^{15}\text{N}$  excess measurements suggest that maximum  $^{15}\text{N}$  uptake occurred in the first 4 m of the buffer areas and the overall uptake was less than that of the pasture. A conservative estimate is that approximately 3% of the  $^{15}\text{N}$  applied to the buffered treatments was taken up in the first 4 m. Only 1 to 2% of the applied  $^{15}\text{N}$  was stored in the upper 15 cm of the soil in the first 5 m of the buffer.

Given the downslope movement of soil  $^{15}\text{N}$  (Fig. 5), the expected pattern of plant  $^{15}\text{N}$  uptake was a gradual increase in plant  $^{15}\text{N}$  further downslope with each subsequent irrigation. Neither the grasses nor the verbena clearly demonstrated this pattern (Fig. 4), instead  $^{15}\text{N}$  enrichment decreased slightly as the vegetation took up non-enriched N. There are two possible explanations for this. The first, supported by the runoff data, is that the majority of downslope  $^{15}\text{N}$  movement was in the less plant-available DON form, so even though N is present, the plants cannot readily access it. The second is that the vegetation within the buffer was no longer taking up N. Maximum N uptake varies with the N status of the vegetation,  $\text{NO}_3^-$  availability, and plant age. Plant uptake tends to decrease with plant age, which may be related to relative growth (Schenk, 1996). As Jackson et al. (1988) observed in the annual grasslands at the Sierra Foothill Research and Extension Center, even well-watered grasses can senesce within weeks of anthesis.

### Buffer Sustainability

The ability of these buffers to remove new N stands in contrast to earlier findings by Tate et al. (2000b) that buffers are ineffective in reducing  $\text{NO}_3^-$  concentrations in irrigated pasture. Although the buffers were effective over the course of the summer, the effectiveness varied in the first few weeks following tracer application. Runoff  $\text{NO}_3^-$ - $^{15}\text{N}$  was high in the first irrigation, but quickly decreased with subsequent irrigation; data indicate that  $\text{NO}_3^-$  was just being cycled into the other N pools. Within one day of application, some of the  $\text{NO}_3^-$ - $^{15}\text{N}$  had already been transformed into  $\text{NH}_4^+$  or DON, as shown by measurable levels of excess  $^{15}\text{N}$  in these forms. Hill (1996) found that in most riparian buffer studies, loss of  $\text{NO}_3^-$  was not associated with increased  $\text{NH}_4^+$  or DON, but these studies may be failing to recognize the importance of N cycling. By using stable  $^{15}\text{N}$  isotopes to examine nitrification rates in the annual grasslands at the Sierra Foothill Research and Extension Center, David-

son et al. (1990) showed that although the size of the  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools remains relatively constant over time, they turn over about once a day. They also showed that microbial assimilation of  $\text{NO}_3^-$  occurs at rates similar to those for plant uptake, indicating that microbial assimilation of  $\text{NO}_3^-$  is of much greater importance than previously recognized. The path of the  $^{15}\text{N}$  over the course of the summer indicates the rapid microbial immobilization of a portion of the applied  $^{15}\text{N}$  and its subsequent mineralization and nitrification contributes to the steady low levels of  $^{15}\text{N}$  that continue to be released from the buffers over the course of the summer.

This re-release of  $^{15}\text{N}$  that had previously been sequestered into the organic and inorganic N pools has contributed to the observation that buffers seem to decrease in effectiveness as more runoff events occur (Barling and Moore, 1994; Dosskey, 2002). As an example, the lower effectiveness of the 16-m buffer for attenuating DON may be attributed in part to the buffer itself acting as a substantial source for N (Dillaha et al., 1989; Mendez et al., 1999). As  $^{15}\text{N}$  that was initially stored in the soil beneath the pasture and buffer was gradually transferred downslope via surface and subsurface water movement (Fig. 5), the 16-m buffer had greater area for  $^{15}\text{N}$  to be stored initially, but its sequestration was transient. With subsequent irrigations, and particularly later in the irrigation event when runoff levels were at their maximum, more DON was released and transported in runoff. Similarly, the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were mineralized from the DON pool and mobilized via runoff during subsequent irrigation events. The  $\text{NH}_4^+$  may have been particularly susceptible to nitrification during the dry periods between irrigation events (Barling and Moore, 1994). This pattern of N cycling within the pasture and buffer soils can account for the smaller peak of  $^{15}\text{N}$  released in the leading edge of the runoff during each irrigation event over the summer.

The corresponding decrease in the spring measurements of vegetation  $^{15}\text{N}$  levels with the stable measurement of  $^{15}\text{N}$  soil levels following the winter rainy season indicates that the  $^{15}\text{N}$  that was originally stored in the plants was subsequently returned to the soil via decomposition of plant materials during the cooler weather. Harvesting vegetation may remove sequestered nutrients from the buffer before they can be re-released into the system (Dosskey, 2001). Evidence suggests that within two weeks after the cutting of vegetation, uptake of N will increase due to increased  $\text{NO}_3^-$  uptake and assimilation (Ourry et al., 1990). It may be that the limited ability of these buffers to take up new or old N later in the irrigation season could be improved by managing the buffer to increase demand for N.

As Sabater et al. (2003) observed, there can be a very large range of  $\text{NO}_3^-$  removal efficiencies in buffers when  $\text{NO}_3^-$  inputs are very low; when  $\text{NO}_3^-$  inputs increase to greater than  $5 \text{ mg L}^{-1}$ ,  $\text{NO}_3^-$  removal efficiency can decrease exponentially. Runoff  $\text{NO}_3^-$  load in these irrigated pastures tends to be relatively low ( $<2 \text{ mg L}^{-1}$ ), but increasing  $\text{NO}_3^-$  inputs might result in much lower buffer efficiency.

## CONCLUSIONS

Although net  $^{15}\text{N}$  runoff losses were relatively low (3%), this study is of significance for a greater understanding of buffer function. By examining only new N inputs distinct from the much larger background N pool, this study clearly illustrates that (i) vegetative uptake is a major mechanism for attenuating new N in irrigated pasture systems and (ii) nutrient cycling within vegetative buffers is indeed serving as both a sink and a source for N in runoff.

The majority of the applied  $^{15}\text{N}$  was attenuated via plant uptake within the zone of  $^{15}\text{N}$  application; a smaller percentage was stored in the first few meters of the buffer vegetation. However, without proper planning, the N sequestered in vegetation may be lost to decomposition, resulting in net N losses. To maximize long-term effectiveness and sustainability of buffer, the potential for increasing vegetation demand and uptake through buffer management must be explored.

Over the course of the study, buffers were effective for attenuating  $\text{NO}_3^-$ - $^{15}\text{N}$ , slightly more effective for  $\text{NH}_4^+$ - $^{15}\text{N}$ , and least effective for  $\text{DON}$ - $^{15}\text{N}$ . For  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , the 16-m buffer was slightly more effective than the 8-m buffer, probably due to greater potential for plant N uptake. Nitrogen cycling within the soil was probably the major source of runoff mineral N later in the season. For DON, the 16-m buffer was actually less effective than the 8-m buffer, indicating that the 16-m buffers themselves were serving as a source for this less plant-available form of N.

Nutrients should always be managed first via in-field conservation practices; buffers should only be used as a secondary measure to capture excess. At this site, maximum differences between buffered and nonbuffered plots were observed primarily at the leading edge of irrigation events and in the first few weeks following fertilizer application. Proper timing and management of fertilizer application coupled with improved irrigation practices to decrease runoff could significantly reduce the potential for nutrient losses.

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