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ECOSYSTEM ECOLOGY - ORIGINAL RESEARCH

Nitrogen cycling and water pulses in semiarid grasslands: are microbial and plant processes temporally asynchronous?

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Abstract Precipitation pulses in arid ecosystems can lead to temporal asynchrony in microbial and plant processing of nitrogen (N) during drying/wetting cycles causing increased N loss. In contrast, more consistent availability of soil moisture in mesic ecosystems can synchronize microbial and plant processes during the growing season, thus minimizing N loss. We tested whether microbial N cycling is asynchronous with plant N uptake in a semiarid grassland. Using ^{15}N tracers, we compared rates of N cycling by microbes and N uptake by plants after water pulses of 1 and 2 cm to rates in control plots without a water pulse. Microbial N immobilization, gross N mineralization, and nitrification dramatically increased 1–3 days after the water pulses, with greatest responses after the 2-cm pulse. In contrast, plant N uptake increased more after the 1-cm than after the 2-cm pulse. Both microbial and plant responses reverted to control levels within 10 days, indicating that both microbial and plant responses were short lived. Thus, microbial and plant processes were temporally synchronous following a water pulse in this semiarid grassland, but the magnitude of the pulse substantially influenced whether plants or microbes were more effective in acquiring N. Furthermore, N loss increased after both small and large water pulses (as shown by a decrease in total ^{15}N recovery), indicating that changes in precipitation event sizes with future climate change could exacerbate N losses from semiarid ecosystems.

Keywords ^{15}N tracer · Nitrogen cycling · Plant-microbial interactions · Temporal effects · Water addition

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Introduction

Global climate change is expected to cause more intense and longer droughts in many parts of the world, while at the same time heavy precipitation events are likely to increase (Meehl et al. 2007). Indeed, in western North America, a trend of prolonged periods without precipitation alternated with increasing precipitation intensity has already emerged during the last 40 years (Groisman and Knight 2008). These changes in precipitation pattern will have large consequences for nitrogen (N) cycling and loss, particularly in arid and semiarid systems where the N cycle is strongly linked to drying/wetting cycles (Austin et al. 2004; Saetre and Stark 2005; Borken and Matzner 2009). Biological activity is largely controlled by water availability in these systems (Noy-Meir 1973; Sala et al. 1988; Huxman et al. 2004a), thereby affecting the supply and demand of N, a limiting nutrient for plant production in most terrestrial ecosystems (Vitousek and Howarth 1991), including arid and semiarid ecosystems (Lauenroth et al.

1978; Hooper and Johnson 1999). There is an increasing awareness that plants and microbes can have different sensitivities to water pulse sizes, that may cause temporal shifts and delays in net N mineralization by microbes and N uptake by plants, with long-term implications for N loss, plant productivity, and soil C sequestration (Austin et al. 2004; Schwinning and Sala 2004; Collins et al. 2008; Austin 2011).

Seasonal N dynamics have been studied in great detail in mesic environments, in both grasslands and forests (e.g., Nadelhoffer et al. 1984; Frank and Groffman 1998; Kaiser et al. 2011; O' Sullivan et al. 2011). In mesic ecosystems, rates of plant growth and microbial activity are often synchronized by seasonal fluctuations in temperature and water availability, characterized by minimal activity during the fall and winter followed by a burst of growth and N mineralization during the spring and summer. As such, microbial N supply is often in concert with plant N demand creating a relatively closed N cycle in unperturbed systems (Bowden et al. 1991; Vitousek et al. 1998; Knops and Tilman 2000; McCulley et al. 2009).

In arid and semiarid ecosystems systems, N supply by microbes and N demand by plants may be discontinuous and temporally asynchronous, due to intense drying/wetting cycles and variation in the time frame over which different processes respond to moisture pulses (Schwinning and Sala 2004; Collins et al. 2008; Austin 2011). Collins et al. (2008) proposed that nutrient supply and demand in arid ecosystems can be described with a Threshold-Delay Nutrient Dynamics (TDND) model. This model predicts that soil microbial processes (such as decomposition and mineralization) and plant nutrient uptake can be uncoupled because the activity of plants and microbes is controlled by different soil moisture thresholds. Small rainfall events can activate microbial processes much more so than plant metabolism (Huxman et al. 2004b; Potts et al. 2006). Similarly, after a rainfall event that is large enough to activate both microbes and plants, the TDND model predicts that metabolism will shut down sooner for plants than for microbes when soils dry out. Continued microbial N mineralization without substantial N uptake by plants could explain why inorganic N sometimes accumulates during extended dry periods (Jackson et al. 1988; Augustine and McNaughton 2004). Because the N supply through microbial processes and N uptake by plants are uncoupled, strong drying/wetting cycles may cause significant N loss through leaching and gaseous pathways (Davidson 1992; McCalley and Sparks 2008; Yahdjian and Sala 2010). Moreover, intense drying/wetting cycles due to human-induced changes in climate could amplify the asynchronicity in N processing between plants and microbes resulting in greater N loss in these systems. Pulsed patterns of microbial and plant N uptake may also have important implications for plant protein content and plant recovery from grazing in these systems where the primary land use is livestock production.

The TDND model was formulated primarily on the basis of research in arid ecosystems characterized by dramatic fluctuations in soil moisture at scales of weeks to months (e.g., Collins et al. 2008). Less is known about the influence of precipitation pulses on the coupling of plant and microbial processes in semiarid ecosystems, although substantial fluctuations in net N mineralization during the growing season have been documented for semiarid grasslands (McCulley et al. 2009; Giese et al. 2011). We studied the temporal pattern of microbial N cycling and plant N uptake following precipitation pulses in a semiarid grassland in Colorado, USA, in order to assess whether dynamics follow the TDND model for arid ecosystems versus the traditional model derived from mesic grasslands and forests. The shortgrass steppe at the USDA-ARS Central Plains Experimental Range where the study was conducted receives on average 340 mm precipitation annually and is prone to large drying/wetting cycles. Precipitation is erratic, with a mean precipitation event size of 1.3 cm and a median of 16 events per growing season (Heisler-White et al. 2008). The TDND model predicts that the water threshold to activate microbial processes (gross N mineralization, gross nitrification, and microbial uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$) is lower than the water threshold to activate plant N uptake (uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$). Based on this model, we developed the following predictions:

1. The increase in microbial N cycling rates following water addition would be the same or larger from the control to the +1 cm treatment than from the +1 to the +2 cm treatment (i.e., a convex relationship between microbial N cycling rates and soil moisture), while the increase in plant N uptake would be larger from the +1 to the +2 cm treatment than from the control to the +1 cm treatment (concave relationship with soil moisture).
2. The increase in microbial N cycling rates would persist longer after a water pulse (still elevated after 8–10 days of drying) than plant N uptake (reverted back to control levels after 8–10 days of drying).
3. Both water pulses would increase N loss as measured by reduced ^{15}N recovery in soil and plant biomass compared to control plots.

Materials and methods

Study site

This experiment was conducted in a native grassland at the USDA-ARS Central Plains Experimental Range, Colorado,

USA. Mean annual precipitation in this grassland is 340 mm, with the majority occurring in May, June, and July. Precipitation is episodic and only falls in 2–4 % of the hours of the year (Pielke and Doesken 2008). Storms with more than 20 mm of water occur <10 % of all precipitation events, but they contribute more than 30 % of annual precipitation. Mean air temperatures are 15.6 °C in July and 0.6 °C in January, while potential evapotranspiration exceeds precipitation by a factor of 3 (Lauenroth and Bradford 2006). In this region, the vegetation is dominated by a mixture of warm-season C_4 [*Bouteloua gracilis* (H.B.K) Lag. and *Bouteloua dactyloides* (Nutt.) J.T. Columbus] and cool-season C_3 grasses [*Pascopyrum smithii* (Rydb.) A. Love, *Hesperostipa comata* Trin and Rupr.]. The site is interspersed with patches of the cactus *Opuntia polyacantha* Haw. In our study plots, bare soil was 29 % of the basal cover. The soil is a well-drained Olney fine sandy loam (fine loamy, mixed, superactive mesic Ustic Haplargid).

Water pulse treatments

In the spring of 2009, we established 15 experimental plots (2 × 2 m) across a 35 × 25 m study area. The study area was within a pasture that had been under moderate summer grazing (stocking rate of 0.8–1.0 ha per animal unit month removing approximately 40 % of annual aboveground plant growth) for >10 years and had no history of tillage, but cattle were excluded from the study area throughout 2009.

We erected rainout shelters over all experimental plots. These shelters measured 2.4 × 2.4 m and 1 m high, thus excluding rainfall from the study plot and a 0.4-m buffer zone around the plot. The shelter roof was clear PVC, and the frame was untreated lumber. Gutters and downspouts diverted incoming rain to an outlet >2 m away from the study plot. In addition, lateral water movement was limited by galvanized steel barriers that extended 15 cm into the soil and 5 cm above it. We effectively diverted rainfall for 21 days from June 24 until the experimental water additions began on July 13. Based on our analysis of the CPER climate record, a 21-day dry period is in the 80th percentile (i.e., 20 % of dry intervals in this part of summer are longer than 21 days).

One centimeter of water was added to five randomly chosen experimental plots (+1 cm), while 2 cm of water was added to another five plots (+2 cm); the other five plots were not watered (control). Water was added between 1230 and 1320 hours on July 13. Water volumes were metered onto each plot using a water flow meter (Omega Scientific, Stamford, CT, USA), and water volumes were adjusted for each plot to account for the slight variations from the targeted 2 × 2 m plot size. The water additions typically took 2–4 min. Although there was ponding in some of the +2 cm treatments, the water infiltrated in <20 min. The experimental water was brought from a nearby groundwater source and was untreated. The +1 and +2 cm treatments were chosen with the assumption that the +1 cm treatment would surpass the water threshold to activate microbial N cycling, while the +2 cm treatment would surpass the water threshold for plant N uptake. Actual pulse thresholds depend on initial soil moisture conditions and length of the dry period preceding the pulse (Schwinning and Sala 2004), but plant growth at our site often does not respond to rainfall events smaller than 1 cm after long dry periods (personal observations).

^{15}N labeling and sampling

One day after the water pulse, we injected ^{15}N into polyvinyl chloride collars (height 15 cm and diameter 10 cm pounded 15 cm into the ground 2 months earlier). Two collars in each plot were injected with a combination of 0.2 g ^{15}N m^{-2} as $K^{15}NO_3$ and 0.2 g ^{14}N m^{-2} as $(^{14}NH_4)_2SO_4$ (total of 0.4 g N m^{-2}), and two collars with a combination 0.2 g ^{15}N m^{-2} as $(^{15}NH_4)_2SO_4$ and 0.2 g ^{14}N m^{-2} as $K^{14}NO_3$ to 10 cm soil depth. With 18-gauge Quincke spinal needles (Becton–Dickinson, Franklin Lakes, NJ, USA) a total of 12 ml was injected in each collar (3 injections of 2 ml each at 2.5 cm and 3 injections of 2 ml each at 7.5 cm soil depth). This amount is similar to a rain event of 0.15 cm and increased the soil moisture content to 10 cm soil depth in all collars by 1.5 % volumetrically or 1.3 % gravimetrically. One of the $K^{15}NO_3$ labeled collars and one of the $(^{15}NH_4)_2SO_4$ labeled collars were harvested directly after the injections, and the remaining two collars after 48 h (3 days after the water pulse). Shoot biomass was clipped in each collar and root biomass was collected by sieving soils sampled to 10 cm depth. We located the collars inside the plots so that the vegetation inside the collars would be predominantly from *B. gracilis* (i.e., more than 95 % of the shoot biomass collected inside the collars was from *B. gracilis*), the most abundant grass at this site. One day after the water pulse, we also collected soil, *B. gracilis* shoot and root biomass from five locations outside of the plots for background ^{15}N analyses (see below). Eight days after the water pulse, a second ^{15}N labeling was initiated in four additional collars in each plot and sampling 8–10 days after the water pulse was conducted in the same way as in the first ^{15}N labeling event. All ^{15}N injections and sampling were done in blocks of three (one control, one +1 cm, and one +2 cm plot at a time).

Separate soil samples (i.e., not labeled with ^{15}N) were taken for analyses of inorganic N (NH_4^+ and NO_3^-). On day 0 (prior to the water pulse), and 1, 2, 3, 4 and 7 days

after the water pulse, 3 soil cores to 10 cm soil depth were taken from each plot (core diameter 5 cm) and bulked.

Processing of samples and analyses

All soil samples were sieved (4 mm). Gravimetric soil moisture content of all ^{15}N labeled soil samples collected 1, 3, 8, and 10 days after the water pulse was measured based on weight loss after drying 30-g subsamples for 2 days at 105 °C.

Sieved soils sampled 48 h after the ^{15}N injections were used to determine microbial biomass N and ^{15}N recovery (fumigation extraction; Bruulsema and Duxbury 1996). We added 60 mL of 0.05 M K_2SO_4 to a 30-g subsample (nonfumigated sample) and to another 30-g subsample after fumigation with chloroform for 5 days in a vacuum desiccator. Samples were shaken for 1 h and filtered through pre-leached filter paper (Whatman No. 1). The extracts were analysed for total N on a total organic carbon (TOC) analyzer with an N measuring unit attached (Shimadzu TOC-VCPN; Shimadzu Scientific Instruments, Wood Dale, IL, USA). The extracts were also analyzed for ^{15}N on a mass spectrometer (20–20 Stable Isotope Analyzer; Europa Scientific, Chesire, UK) after first freeze-drying aliquots of the extracts. We calculated microbial N as the difference between N in the fumigated and nonfumigated samples divided by 0.54 (Brookes et al. 1985). We calculated the ^{15}N atom% in microbial biomass ($^{15}N_{mic}$) using:

$$^{15}N_{mic} = (^{15}N_f \times N_f - ^{15}N_e \times N_e)/(N_f - N_e) \quad (1)$$

where $^{15}N_f$ and N_f are the ^{15}N atom% and total amount of N in the fumigated extracts, $^{15}N_e$ and N_e the ^{15}N atom% and total amount of N in the nonfumigated extracts. We calculated ^{15}N recovery in the microbial N pool in the ^{15}N labeled collars ($^{15}N_{rec,mic}$) using:

$$^{15}N_{rec,mic} = N_{mic,l} \times \left(^{15}N_{mic,l} - ^{15}N_{mic,n}\right)/\left(^{15}N_{label} - ^{15}N_{mic,n}\right) \quad (2)$$

where $N_{mic,l}$ and $^{15}N_{mic,l}$ are the total amount of N and ^{15}N atom% in the microbial biomass labeled with ^{15}N, $^{15}N_{mic,n}$ the average ^{15}N atom% in the microbial biomass not labeled with ^{15}N (average of the five off-plot samples), and $^{15}N_{label}$ the ^{15}N atom% of the label.

Sieved soils sampled directly and 48 h after ^{15}N injection were extracted for $^{15}NO_3^-$ and $^{15}NH_4^+$ analyses to determine gross N mineralization and gross nitrification rates (pool dilution method; Kirkham and Bartholomew 1954). In brief, 60 mL of a solution of 1 M KCl was added to a 30-g subsample, shaken for 1 h, and filtered through pre-leached filter paper (Whatman No. 1). The KCl extracts were analyzed for NH_4^+ and NO_3^- concentrations on a flow injection analyser (QuikChem FIA+; Lachat Instruments, Milwaukee, WI, USA). For the soils labeled with ^{15}N as $(NH_4)_2SO_4$, the NH_4^+ in the KCl extracts was collected using acidified filter-paper disks inside PTFE diffusion traps (Stark and Hart 1996). For the soils labeled with ^{15}N as KNO_3, NH_4^+ was first removed by adding MgO and letting the samples sit for 5 days. Then, Devarda's alloy was added to convert the NO_3^- into NH_4^+, which was collected as above. The filter disks were analyzed for total N and ^{15}N atom% on a mass spectrometer.

Non-labeled soil samples taken just before the water pulse (day 0) and 1, 2, 3, 4, and 7 days after the water pulse were processed and analyzed for extractable NH_4^+ and NO_3^- with KCl as described above. Inorganic N pools, microbial N pools, rates of gross N mineralization, and gross nitrification, and ^{15}N recoveries were expressed per m^2 using bulk densities calculated from the total amount of soil collected inside the collars.

Root biomass collected after 48 h was washed and, together with shoot biomass, dried (60 °C) and weighed. Ground plant and soil samples were analyzed for total N and ^{15}N on a mass spectrometer. We calculated ^{15}N recovery in total soil, shoot, and root biomass as we did for microbial biomass with Eq. 2. Total ^{15}N recovery was calculated as the sum of the ^{15}N recoveries in plant biomass and soil.

We used ANOVA to test for water (control, +1 cm, +2 cm), ^{15}N form (NH_4^+ and NO_3^-), and date effects on gravimetric soil moisture (%), plant tissue N concentrations (%), plant and microbial N pools (g m^{-2}), and ^{15}N recovery in the total system, and in individual plant and microbial biomass pools (g m^{-2}). We used a partly nested design with the water treatment as the between-plots factor, the ^{15}N form and date treatments as within-plots factors, and block as a random factor nested within the water treatment (Quinn and Keough 2002). We also used Tukey's HSD tests to compare means of the different water and ^{15}N form treatment combinations for each date. For effects on gravimetric soil moisture, we included the measurements made 1, 3, 8, and 10 days after the water pulse, while for the other variables, measurements on days 3 and 10 after the water pulse were used. For testing treatment effects on extractable NH_4^+, extractable NO_3^- (from non-labeled soils collected 0, 1, 2, 3, 4, and 7 days after the water pulse), gross N mineralization, and gross nitrification (g m^{-2} day^{-1}) we used a similar design but without the ^{15}N form treatment as a within-plots factor. When necessary, data were log-transformed to meet assumptions of normality and to reduce heteroscedasticity. Treatment effects were considered significant at $P < 0.05$. All statistical analyses were done with JMP (v.4.0.4; SAS Institute, Cary, NC, USA).

Table 1 Summary of ANOVA results (*P* values) for the effects of water (control, +1 and +2 cm), ^{15}N form (NH_4^+ and NO_3^-) and date after pulse (*ns* not significant, $P > 0.1$)

Effect	Soil moisture	Microbial N	Gross N miner.	Gross nitrif.	Total ^{15}N rec	Shoot ^{15}N rec	Root ^{15}N rec	Total plant ^{15}N rec	Microbial ^{15}N rec	Extract. NH_4^+	Extract. NO_3^-
Water	<0.0001	0.003	0.006	0.004	0.02	0.04	0.03	0.01	0.04	ns	0.04
^{15}N form	ns	ns	–	–	ns	<0.0001	ns	0.006	ns	–	–
Water × ^{15}N form	ns	ns	–	–	ns	ns	ns	ns	ns	–	–
Date	<0.0001	<0.0001	<0.0001	0.0001	ns	0.0009	0.03	<0.0001	0.02	ns	ns
Date × water	0.0003	0.0007	0.003	0.03	ns	ns	ns	ns	0.03	0.02	ns
Date × ^{15}N form	ns	ns	–	–	ns	0.003	0.005	ns	ns	–	–
Date × water × ^{15}N form	ns	ns	–	–	ns	ns	ns	ns	0.09	–	–

^{15}N form was not included in the ANOVA for gross N mineralization, gross nitrification, extractable NH_4^+, and extractable NO_3^-

Results

The addition of 1 and 2 cm of water caused significant effects on gravimetric soil moisture, where soil moisture was about 7 and 8 % higher in the top 10 cm of the soil with the addition of 1 and 2 cm of water, respectively (Table 1; Fig. 1). Soil moisture in the +1 and +2 cm water treatments declined rapidly with time, and 10 days after the water pulse soil moisture reached a similarly low level as in the control plots. Soil moisture did not differ between N form treatments (Table 1).

We observed no significant main or interactive effect of water, ^{15}N form, and date on plant tissue N concentrations or plant N pools (Table S1, Electronic Supplementary Material). However, the water treatments caused dramatic increases in microbial N pools 3 days after the water pulse, with increases of 1.1 and 2.7 g N m^{-2} (or 71 and 170 %) in the +1 and +2 cm water treatments, respectively, compared to the control plots (Fig. 2a). Note that these increases were much larger than the 0.4 g N m^{-2} applied to the collars. However, these increases disappeared completely after 10 days. No significant main or interactive effects of ^{15}N form on microbial N were observed (Table 1). Gross mineralization and gross nitrification showed very similar temporal responses to the water treatments. While gross mineralization in the control plots was close to zero (0.02 g m^{-2} day^{-1}), it increased to 0.16 and 0.22 g m^{-2} day^{-1} in the +1 and +2 cm water treatments, respectively, after 3 days, and returned to virtually negligible rates after 10 days (Fig. 2b). Gross nitrification increased from 0.05 g m^{-2} day^{-1} in the control plots to 0.22 and 0.36 g m^{-2} day^{-1} in the +1 and +2 cm plots, respectively, after 3 days. After 10 days, gross nitrification rates were near zero in the control and +1 cm treatment and fell back 0.07 g m^{-2} day^{-1} in the +2 cm treatment (Fig. 2c).

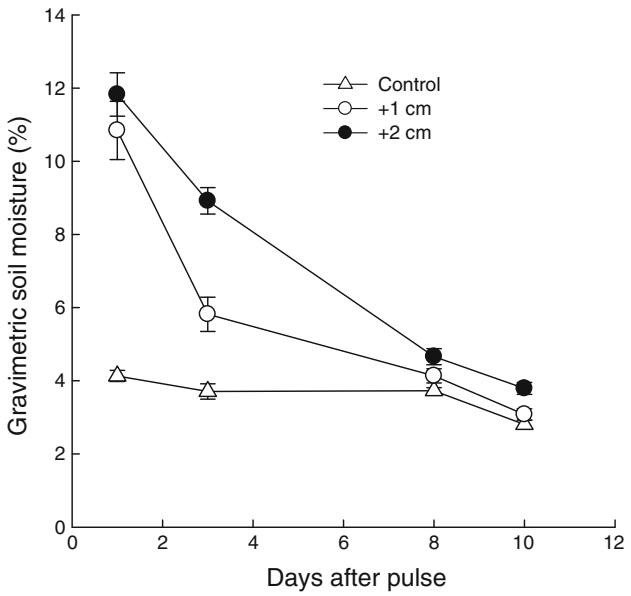

Fig. 1 Average gravimetric soil moisture after initiation of water treatments (days after pulse) for plots receiving no water addition (*control*), a 1 cm (+1 cm), and a 2 cm water pulse (+2 cm) averaged across ^{15}N form. *Error bars* 1SE

While none of the treatments significantly altered plant N concentrations or pools (Table S1, Electronic Supplementary Material), water, ^{15}N form and date had significant effects on ^{15}N recovery in plant biomass (Table 1). ^{15}N Recovery in shoot and root biomass increased with water addition. The ^{15}N recovery in plant biomass was higher in the +1 than in the +2 cm treatment during 1–3 days after the pulse (Fig. 3a–c). Eight to ten days after the pulse, ^{15}N recovery in plant biomass was lower compared to the 1–3 days measurement, but still significant (Table 1). ^{15}N Recovery in shoot biomass was greater where ^{15}N was added as NO_3^- than as NH_4^+. ^{15}N Recovery after 3 days in root biomass on the other hand was greater with $^{15}NH_4^+$ than with $^{15}NO_3^-$ addition (Fig. 3b). After 10 days, this pattern in roots reversed to greater ^{15}N recovery with

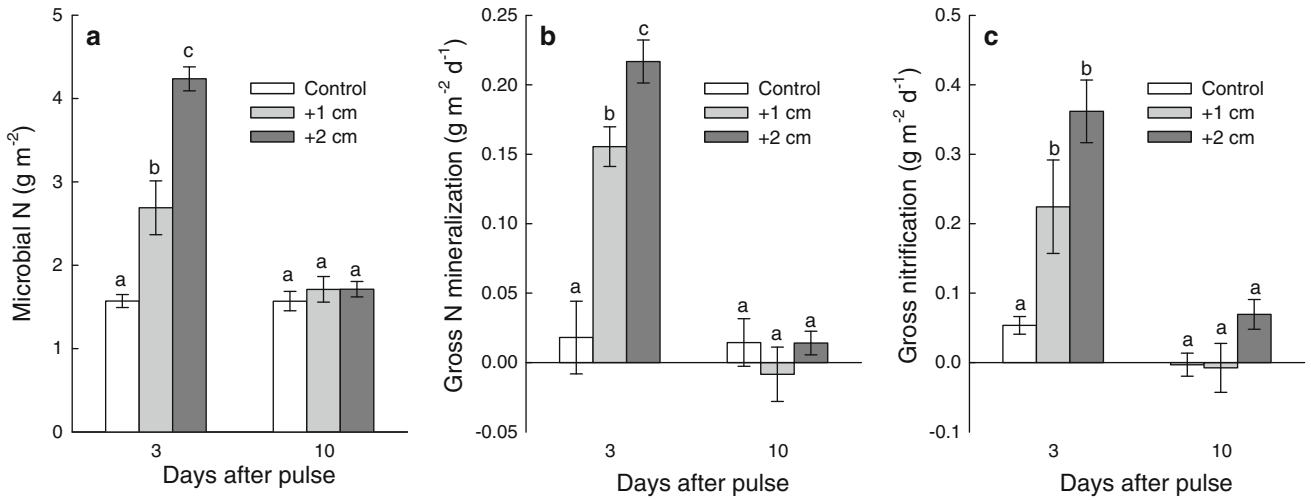

Fig. 2 Average microbial N pool averaged across ^{15}N form (**a**), gross N mineralization (**b**), and gross nitrification (**c**) 3 and 10 days after initiation of water treatments (days after pulse) for plots receiving no water addition (*control*), a 1 cm (+1 cm), and a 2 cm water pulse (+2 cm). *Error bars* 1SE. *Different letters* above *bars* indicate significant differences among water treatments for each date separately ($P < 0.05$, Tukey's HSD test)

$^{15}NO_3^-$ addition. Total plant ^{15}N recovery was higher with the $^{15}NO_3^-$ than with the $^{15}NH_4^+$ addition in all water and date treatments (Fig. 3c). Similar to plant biomass, ^{15}N recovery in microbial biomass increased 3 days after water addition, but unlike ^{15}N recovery in plants biomass, ^{15}N recovery in microbial biomass was greatest in the +2 cm water treatment (Fig. 3d).

Both water pulses caused increases in extractable NH_4^+ 1 day after the pulse that reverted back to control levels after 2 days (significant date × water interaction; Table 1; Fig. 4). Extractable NO_3^- was generally lower than extractable NH_4^+. Extractable NO_3^- significantly increased with water addition (Table 1), which peaked 2 days after the pulse and reverted back to control levels after 7 days (Fig. 4).

Of the 200 mg m^{-2} of ^{15}N that was added to the collars 1 and 8 days after the pulse, 216 ± 21 and 227 ± 16 mg m^{-2} (average across ^{15}N form ±standard error) was recovered in the control plots after 3 and 10 days, respectively, but in the +1 and +2 cm treatments only 157 ± 21 and 151 ± 15 mg m^{-2}, respectively (or 79 and 76 % of added ^{15}N), was recovered after 3 days (of the first ^{15}N injection), and 184 ± 13 and 168 ± 11 mg m^{-2}, respectively (or 92 and 84 % of added ^{15}N), after 10 days (of the second ^{15}N injection). While the total ^{15}N recovery significantly decreased with water addition, main and interactive effects of date and ^{15}N form were not significant (Table 1).

Discussion

Our findings indicate that this semiarid grassland does not conform to the TDND model (Collins et al. 2008). Although both plants and microbes responded to the water pulses after a 21-day dry period in terms of increased microbial N content, gross N mineralization, gross nitrification, and plant and microbial ^{15}N recovery, we expected that plant ^{15}N recovery would respond much stronger to the +2 cm treatment than to the +1 cm treatment (first prediction). Instead, ^{15}N uptake by plants was smaller in the +2 cm pulse than in the +1 cm treatment. We also expected that microbial N cycling responses would persist longer than plant ^{15}N uptake responses (second prediction). Instead, 10 days after the water pulse, ^{15}N recovery in microbial biomass was close to zero and not different from ^{15}N recovery in the control treatment, while uptake of ^{15}N by plant biomass was still significant (particularly as NO_3^-), including in the control treatment. This could potentially be related to microbes in 0–10 cm soils being directly affected by moisture in that layer, whereas plants still had access to moisture in deeper soil layers that could help sustain their activity and N uptake over a longer time period. However, both plant and microbial N cycling rates declined rapidly between 1–3 versus 8–10 days after the pulses, suggesting that, for the soil moisture conditions we studied, the shortgrass steppe is better characterized by synchronous plant and microbial N processing typical of more mesic grasslands (McCulley et al. 2009).

Microbes were strongly limited by soil moisture in this grassland and showed large immediate responses to the water pulse that disappeared when soil moisture returned to the low level in the control treatment. Large but short-lived increases in microbial N pools, gross N mineralization, and gross nitrification after rainfall events were also observed in other arid and semiarid ecosystems (Billings et al. 2004; Saetre and Stark 2005; Ford et al. 2007). Soil extractable

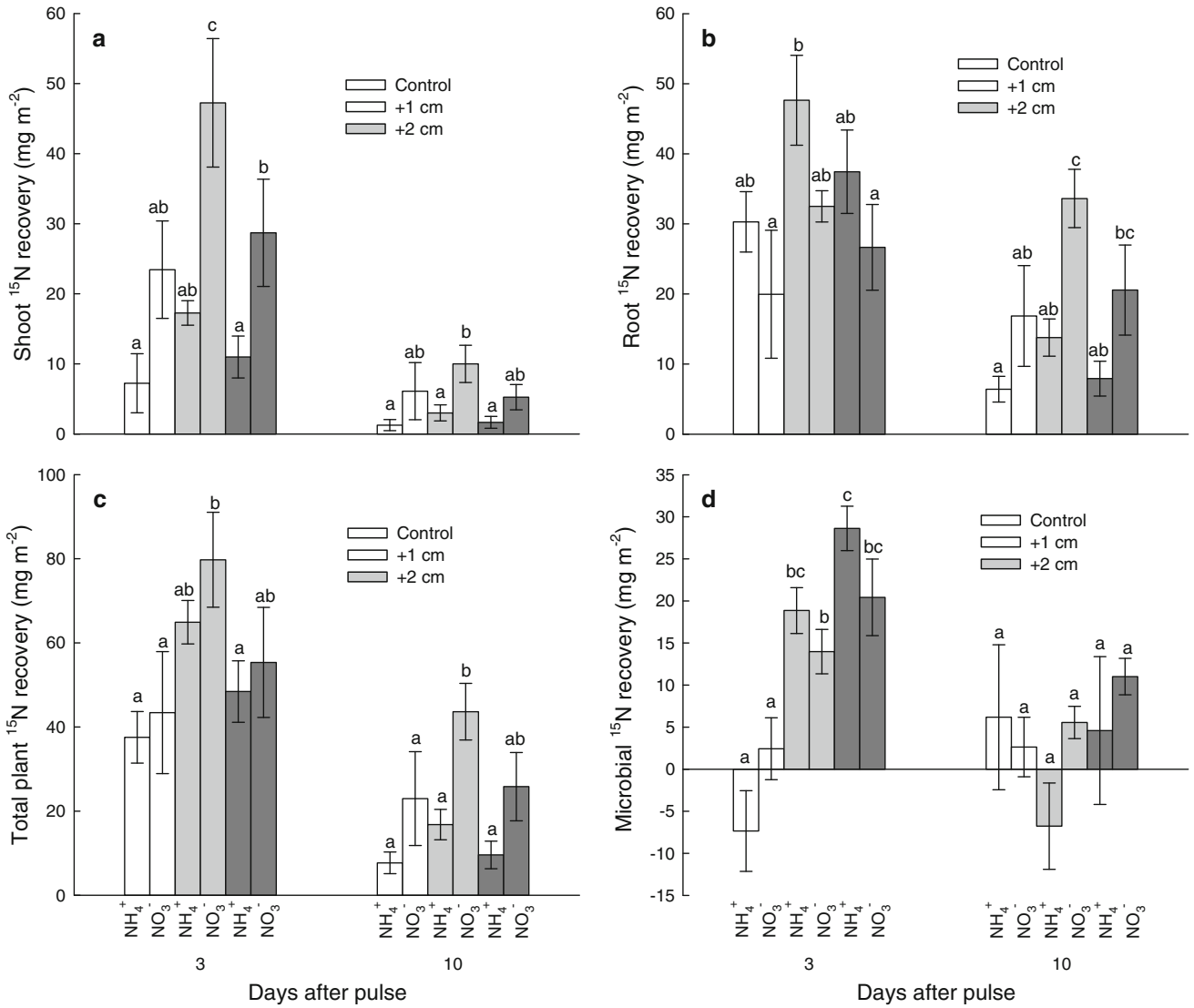

Fig. 3 Average ^{15}N recoveries in shoot biomass (**a**), root biomass (**b**), total plant biomass (**c**), and microbial biomass (**d**) 3 and 10 days after initiation of water treatments (days after pulse) for plots receiving no water addition (*control*), a 1 cm (+1 cm), and a 2 cm water pulse (+2 cm) and receiving ^{15}N either as NH_4^+ or as NO_3^-. *Error bars* 1SE. *Different letters* above *bars* indicate significant differences among water and ^{15}N-form treatments for each date separately ($P < 0.05$, Tukey's HSD test)

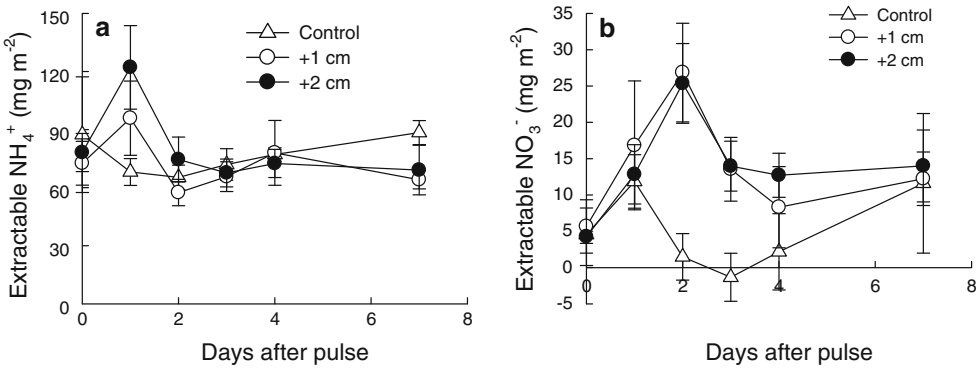

Fig. 4 Average extractable NH_4^+ (**a**) and NO_3^- (**b**) in soils unamended with ^{15}N just before (day 0) and after initiation of water treatments (days after pulse) for plots receiving no water addition (*control*), a 1 cm (+1 cm), and a 2 cm water pulse (+2 cm). *Error bars* 1SE

NH_4^+ peaked at 1 and NO_3^- at 2 days after the water pulse and returned to control levels after 2 and 7 days, respectively. All microbial N cycling parameters responded strongly to the +1 cm treatment suggesting that the soil moisture threshold for microbes to become active lies below rain events of 1 cm. On the other hand, microbial

^{15}N recovery and N cycling rates remained close to zero in the control treatment even though this treatment received 0.15 cm of water with the ^{15}N injections, suggesting that the soil moisture threshold for microbial processing of N is above 0.15 cm. Our results show that microbial effects on N cycling in the shortgrass steppe are sensitive to relatively small water increases after a dry period, but that they are short lived, which contrasts reports of continued microbial processing of N during extended dry periods in other arid and semiarid ecosystems (Singh et al. 1989; Augustine and McNaughton 2004; Collins et al. 2008). Variable responses to water pulses among different systems may have occurred because of differences between temperate and tropical systems where temperate systems fluctuate both in water availability and temperature while tropical systems (Singh et al. 1989; Augustine and McNaughton 2004) vary mostly in water availability. Variable responses may also have occurred because of differences in microbial composition (Collins et al. 2008), and variation in length and severity of dry periods preceding a moisture pulse (Schwinning and Sala 2004). The rapid response and shutdown of microbial N processing that we observed after water pulses shows that water availability in this semiarid grassland is crucial to mechanisms and pathways of N availability and loss.

In the semiarid shortgrass steppe, large, infrequent precipitation pulses have been shown to promote aboveground plant growth to a greater degree than smaller, more frequent pulses (Heisler-White et al. 2008), but effects of pulse size on plant N acquisition have not been evaluated. We found that plant ^{15}N uptake responded strongly to soil moisture (+1 cm pulse), but plant ^{15}N uptake was lower following the +2 cm versus the +1 cm pulse, suggesting that plants were more limited by inorganic N availability following the +2 cm pulse. This finding was surprising because the addition of +2 cm of water increased soil moisture up to 12 % (Fig. 1), which is well below field capacity (~30 % soil moisture). Thus, soil moisture levels in the +2 cm treatment were not high enough to inhibit biological activity. We suggest that lower plant ^{15}N recovery following the larger water pulse was caused by increased competition for inorganic N by microbes in the wetter soils. Plant–microbe competition for inorganic N has been suggested as a mechanism that can control N availability to plants (Kaye and Hart 1997; Bontti et al. 2011; Xu et al. 2011). Microbial N immobilization rate may have increased more than the rate of gross N mineralization in the +2 cm treatment 1–3 days after the water pulse when microbial N immobilization (or microbial ^{15}N recovery) was still high, thereby reducing available N to plants compared to the +1 cm treatment. The sharp decrease in microbial ^{15}N recovery 10 days after the water pulse suggests that microbial competition for N also declined. However, water addition effects on net N mineralization that we did not directly measure may still have contributed to the larger plant biomass ^{15}N recovery in the +1 cm treatment compared to the +2 cm treatment after 10 days. In one of the few studies where the size of water pulses on N dynamics were examined, net N mineralization rates were also greater in small events compared to large events (Yahdjian and Sala 2010). Our findings combined with those of Heisler-White et al. (2008) and Yahdjian and Sala (2010) suggest that, in semiarid grasslands, precipitation pulses that maximize short-term aboveground plant production rates are larger than pulses that optimize plant N acquisition.

We observed that plant uptake of $^{15}NO_3^-$ was greater than uptake of $^{15}NH_4^+$. Plant uptake of inorganic N from the soil is often limited by the rate of diffusion through the soil, which declines when soils dry (Nye 1977). Because NO_3^- has greater mobility than NH_4^+ in soil (i.e., the diffusion coefficient is larger for NO_3^- than for NH_4^+; Lambers et al. 2008), plant access to $^{15}NO_3^-$ was greater than access to $^{15}NH_4^+$. Interestingly, the greater uptake of $^{15}NO_3^-$ by plants was not affected by the water pulse treatment (no water × ^{15}N form interactive effect on ^{15}N recovery in plants; Table 1), suggesting that potential increases in diffusion rates after soil wetting did not alter the relative uptake of NH_4^+ and NO_3^- by plants. Actual uptake of NH_4^+ by plants may have been higher than actual uptake of NO_3^- because concentrations of NH_4^+ were always higher than NO_3^- before and during the 7-day period after the water pulse (2–18 times higher, while we added $^{15}NH_4^+$ and $^{15}NO_3^-$ in equal amounts). The greater abundance of NH_4^+ compared to NO_3^- in the soil after the water pulse may further have caused a disadvantage to plants competing for N with microbes, since microbes showed a slight, although statistically non-significant, preference for $^{15}NH_4^+$ (Fig. 3d).

Our findings also have important implications for grass–grazer interactions in this semiarid ecosystem where grazing is the primary land use. N acquisition is particularly important for plants that have recently lost leaf tissue to grazers. When a large precipitation pulse (preceded by drought conditions) was simulated in a greenhouse study, Augustine et al. (2011) found that microbial biomass N and ^{15}N were similar in both defoliated and non-defoliated microcosms. Furthermore, defoliated *B. gracilis* plants were only able to slightly increase ^{15}N uptake relative to non-defoliated plants (Augustine et al. 2011). Based on our field study, where we observed increased plant–microbe competition for N following large water pulses, loss of plant tissue and N to grazers could exacerbate N limitation to plant growth after large precipitation events. In contrast, smaller precipitation pulses support reduced aboveground plant production with greater relative plant N acquisition rates and less N limitation to plant growth. Future studies

are needed to explicitly test interactions between pulse size, the timing of grazing, and rates of plant recovery from grazing.

In spite of the rather synchronous responses of plant N uptake and microbial processing of N to the water pulse, substantial loss of ^{15}N occurred after the water pulse (supporting our third prediction). We have no direct measurements of N loss (e.g., no measurements of N leaching or gaseous N losses), but the reduced ^{15}N recovery in the +1 and +2 cm treatments suggest a rapid loss of N after a water pulse. Inorganic N leaching was most likely small because even with the +2 cm water pulse soil moisture never reached field capacity, and no large macropores were visible at the site eliminating the possibility of N loss through preferential flow. Because we added the ^{15}N label 1 day after the water pulse addition, we missed N loss that may have occurred in the time period between the water and ^{15}N labeling. Large increases in gaseous N emissions have been observed within 24 h after a pulse of water (Davidson 1993; Norton et al. 2008). The size of the pulse had no effect on total ^{15}N recovery. We further observed the loss of ^{15}N added on day 8 after the water pulse, although soil moisture levels at this time had almost converged to the soil moisture level in the control plots (Fig. 1). It is unclear what caused the loss of ^{15}N 8–10 days after the water pulse. Possibly hysteresis effects on denitrification during wetting and drying of the soil (Groffman and Tiedje 1988) may have extended gaseous N loss after the water pulse, despite similarly low soil moisture levels as in the control plots.

We conclude that the TDND model developed by Collins et al. (2008) does not describe N cycling in this semiarid grassland. Microbes involved in N cycling did not become active at lower soil moisture thresholds than plants, and microbes did not remain active longer after the water pulse. Instead, water pulses had similar temporal effects on plant and microbial activity, with increased microbial N cycling and plant N uptake immediately after the pulse that faded away when soils dried out again. However, the size of the water pulse influenced ensuing N acquisition by plants and microbes in different ways. Microbial N immobilization increased with the size of the water pulse, while plant N uptake increased more after the +1 cm than after the +2 cm water pulse, suggesting that water pulse size could influence the degree of N limitation. Our results further support the idea that water pulses stimulate N loss in this semiarid grassland (McCulley et al. 2009). The sudden and large effects of water pulses on microbial and plant activity increased the rate of net inorganic N release more than the rate of plant inorganic N uptake, causing a short pulse in soil inorganic N and N loss. This N loss may worsen with more severe droughts and more intense precipitation events. An increase in atmospheric N deposition could possibly accelerate N losses after water pulses, but it may also restore some of the N loss incurred after water pulses. Because this grassland and similar semiarid ecosystems are N-limited (Lauenroth et al. 1978; Hooper and Johnson 1999), alterations in the N dynamics may severely impact plant productivity and community structure, forage quality and C sequestration in the long term.

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