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HIGHLIGHTED STUDENT RESEARCH

Increased rainfall variability and N addition accelerate litter decomposition in a restored prairie

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Abstract Anthropogenic nitrogen deposition and projected increases in rainfall variability (the frequency of drought and heavy rainfall events) are expected to strongly influence ecosystem processes such as litter decomposition. However, how these two global change factors interact to influence litter decomposition is largely unknown. I examined how increased rainfall variability and nitrogen addition affected mass and nitrogen loss of litter from two tallgrass prairie species, *Schizachyrium scoparium* and *Solidago canadensis*, and isolated the effects of each during plant growth and during litter decomposition. I increased rainfall variability by consolidating ambient rainfall into larger events and simulated chronic nitrogen deposition using a slow-release urea fertilizer. *S. scoparium* litter decay was more strongly regulated by the treatments applied during plant growth than by those applied during decomposition.

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This manuscript is being submitted for consideration as a highlighted student paper, as its author and progenitor is a graduate student. The manuscript addresses a novel and understudied aspect of global change ecology, namely the interaction of anthropogenic nitrogen and increased rainfall variability to affect prairie litter decomposition, and utilizes an innovative design to illuminate the mechanisms responsible for the observed changes in ecosystem processes.

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During plant growth, increased rainfall variability resulted in *S. scoparium* litter that subsequently decomposed more slowly and immobilized more nitrogen than litter grown under ambient conditions, whereas nitrogen addition during plant growth accelerated subsequent mass loss of *S. scoparium* litter. In contrast, *S. canadensis* litter mass and N losses were enhanced under either N addition or increased rainfall variability both during plant growth and during decomposition. These results suggest that ongoing changes in rainfall variability and nitrogen availability are accelerating nutrient cycling in tallgrass prairies through their combined effects on litter quality, environmental conditions, and plant community composition.

Introduction

Precipitation regimes are expected to become more variable as surface air temperatures rise, increasing the prevalence of prolonged drought and heavy rainfall in many regions of the world (Stocker et al. 2014). Changes in the frequency of these extreme climatic events can significantly alter the global carbon (C) and nitrogen (N) cycles by influencing the decomposition of organic tissues (Jentsch et al. 2011; Reichstein et al. 2013). Similarly, other anthropogenic changes in resource availability, particularly nitrogen, can directly affect C and N cycling by altering terrestrial litter decomposition (Aerts 1997; Vitousek et al. 1997). However, while the influences of climate and soil fertility on litter decomposition have been the focus of ecological research for decades (Swift et al. 1979; Attiwill and Adams

1993; Knorr et al. 2005), little is known about how a climate change-induced increase in rainfall variability will interact with anthropogenic N deposition to regulate the formation and decomposition of litter.

Although a few studies have examined the impacts of increased rainfall variability on ecosystem processes (Jentsch et al. 2011; Beier et al. 2012), longer dry periods and heavier rainfall are expected to increase drought stress in mesic systems (Knapp et al. 2008), which can strongly influence leaf litter quality. Indeed, some tallgrass prairie species produce less biomass under increased rainfall variability, consistent with the expectation of drought stress (Fay et al. 2003). Therefore, plant traits positively associated with productivity, like litter quality, might be expected to be reduced under increased rainfall variability as they are under drought (Fay et al. 2002). While drought does not always directly affect litter quality (Liao et al. 2002; Walter et al. 2013), drought in mesic systems can either increase or decrease litter C:N (Sardans et al. 2012). Drought can lead to higher litter C:N (lower litter quality) in many species by reducing soil fertility and inhibiting leaf productivity (Rennenberg et al. 2009)—a response more common in arid systems, but also observed in mesic systems (Sardans et al. 2012). Since litters with high C:N ratios typically decompose and lose N more slowly than those with low C:N (Melillo et al. 1982; Aerts 1997; Parton et al. 2007), litter of some species might therefore decompose more slowly under future, more variable, precipitation regimes. However, many other plants produce leaves with higher N concentrations (and, thus, lower C:N) under drought as a method of increasing water use efficiency (Cornwell et al. 2007; Weih et al. 2011; Sardans et al. 2012). Leaves with higher N concentrations typically also result in litter with higher N concentrations, and therefore drought during plant growth can also promote higher litter quality (Sardans et al. 2008), accelerating subsequent decomposition. Considering the wide range of observed positive and negative responses of litter chemistry to drought, litter chemistry under increased rainfall variability is likely to be context-specific.

More variable rainfall regimes can directly affect the decomposition process via changes in soil moisture and microbial activity, either hastening or slowing decomposition independently of the impacts of increased rainfall variability on plant growth and litter quality. Decomposition can be reduced for months following the rapid drying and rewetting associated with increased rainfall variability as a result of stifled enzymatic and microbial activity (Fierer et al. 2003; Schimel et al. 2007). Additionally, drought commonly decreases decomposition rates by inhibiting microbial activity and limiting nutrient leaching (Bloor and Bardgett 2012), and extreme rainfall variability has been tied to slower decomposition of several grassland species (Walter et al. 2013). However, increased rainfall variability can also enhance decomposition rates if more frequent heavy rainfall events and prolonged dry-down periods keep soils wetter for longer than they would be under historical conditions (Anaya et al. 2012; Jin et al. 2013). Because increased rainfall variability can enhance or inhibit decomposition via both litter quality and the environmental conditions that mediate litter decay, the implications of projected changes in rainfall for litter dynamics remain poorly understood.

Chronic atmospheric N deposition also strongly influences the production and decomposition of leaf litter. Increased soil N availability promotes higher quality leaf litter by increasing tissue N concentrations (Stump and Binkley 1993; Smith and Bradford 2003; Valera-Burgos et al. 2013), and consequently promotes faster litter decay. However, the direct effects of soil N fertility on the decomposition process are more context-dependent and do not necessarily work in concert with changes in litter quality to accelerate decomposition rates (Knorr et al. 2005; Hobbie 2008). Nitrogen addition has been shown to both accelerate (Manning et al. 2008) and inhibit decomposition (Tu et al. 2014), and to act on varying timescales (Hobbie 2008). These contrasting results may be explained by differences in the effects of N addition on the production of decomposer enzymes. Whereas N addition can enhance cellulase (enzymes that help break down cellulose) activity, it has also been shown to inhibit the production of ligninolytic enzymes, thus favoring the decomposition of low-lignin substrates and inhibiting decomposition of high-lignin substrates (Carreiro et al. 2000; Knorr et al. 2005). Thus, N deposition can exacerbate differences in decomposition rates between typically fast-decomposing litters (low-lignin, low C:N substrates) and typically slow-decomposing litters (high-lignin, high C:N substrates). The sensitivity of low- and high-C:N litters to N addition is also partially regulated by the magnitude of N deposition relative to historical N availability (Knorr et al. 2005). These differences in sensitivity lead to greater differences in decomposition between litters of varying quality in naturally low-fertility soils (in which even low rates of N deposition constitute a large change) or under heavy N deposition.

The effects of increased rainfall variability on litter production and decomposition may depend on or interact with soil fertility and N deposition. Under drought, soil N mobility is reduced and plant access to N is inhibited (Everard et al. 2010). Therefore, many of the consequences of N deposition for plants and microbes are dependent on soil moisture (Knapp et al. 1998; Everard et al. 2010). Thus, litter chemistry and decomposition rates may not respond as strongly to N addition under the drier conditions associated with increased rainfall variability. Conversely, N addition may be more impactful during the wet periods following heavy rainfall events that are also associated with increased

rainfall variability. However, despite their potentially important role in regulating nutrient cycling and ecosystem function, the combined influence of increased rainfall variability and N addition on litter decomposition remains largely unknown. Here, I consider the impacts of increased rainfall variability and N addition on litter dynamics in a restored tallgrass prairie, addressing three guiding research questions: (1) How do increased rainfall variability and N addition during plant growth, separately or in combination, alter litter quality? (2) How do increased rainfall variability and N addition during litter decomposition, separately or in combination, influence litter decay? and (3) Do the effects of increased rainfall variability and N addition during litter decomposition change depending on environmental conditions during litter formation?

Materials and methods

Rainfall and nitrogen manipulations

This experiment took place within the Prairie Invasion and Climate Experiment (PRICLE; Online Resource 1), located within a restored tallgrass prairie at the Purdue Wildlife Area (40.4°N, 86.9°W). PRICLE was established in May 2012 and employed a combination of partial rain-out shelters, watering, and addition of a slow-release polymer-coated urea (ESN; Agrium Advanced Technologies, Loveland, CO, USA) to manipulate rainfall variability and N availability in a fully factorial design (ambient rainfall variability or increased rainfall variability by ambient N or added N) in three blocks of 4 × 5.5 m plots (12 plots total).

Rain-out shelters intercepted and excluded half of all ambient rainfall from plots for 5 months of the 2012 and 2013 growing seasons (May–October). Every 30 days, a volume of water equivalent to the excluded rain was applied, and thus rainfall variability was increased in these plots by consolidating half of all monthly rainfall into a single large event while maintaining cumulative rainfall over the growing season. During the past 111 years, events large enough to double cumulative rainfall over the past 30 days occurred once every other year, on average. However, events of this magnitude occurred five times during the growing season for plots under rain-out shelters, and therefore represent at tenfold increase in the frequency of these heavy rainfall events (Smith et al., unpublished). As a result, increased rainfall variability increased seasonal maximum 5-day cumulative rainfall by 33 % in 2012 and by 9 % in 2013 over ambient conditions—magnitudes comparable to the mean of the CMIP5 climate model ensemble for the year 2100 (Sillmann et al. 2013; Stocker et al. 2014). Plots not under rain-out shelters were covered by structures draped in wildlife netting to control for shading effects.

To simulate atmospherically N-enriched conditions, a polymer-coated urea was applied to plots each year during the first week of June at a rate of 5 g N m^{-2} year^{-1}. This rate of N addition is roughly triple the current total atmospheric deposition rates for the region (National Atmospheric Deposition Program) and is comparable to treatments used in other studies evaluating ecological impacts of N deposition (e.g., BioCon: 4 g N m^{-2} year^{-1}, Reich et al. 2001; NutNet: 10 g N m^{-2} year^{-1}, Borer et al. 2014) as well as N deposition rates in heavily industrialized regions (Vitousek 1994).

Soil moisture

Volumetric soil water content was monitored using 10HS probes (Decagon Devices, Pullman, WA, USA) inserted into the top 10 cm of the soil profile. The effects of increased rainfall variability on soil moisture varied greatly between years, despite total season rainfall remaining constant (Smith et al., unpublished). In 2012, during litter formation, increased rainfall variability increased mean soil moisture, but induced lower soil moisture in periods before the large watering events that took place every 30 days, particularly during the severe drought that occurred during the first half of that year's growing season. In contrast, from the first watering event of the 2013 growing season onward, increased rainfall variability only increased soil moisture compared to ambient conditions.

Litter bag experiment

In order to examine how increased rainfall variability and N addition might affect decomposition and N loss from tallgrass prairie litters, I considered the two dominant species at the site: *Solidago canadensis,* a perennial clonal forb, and *Schizachyrium scoparium*, a perennial C$_4$ bunchgrass. Fully senesced litter from each species was collected from each of the 12 experimental plots in November 2012, after a full season under rainfall and N manipulations. Leaf litter of *S. canadensis* was harvested from standing shoots by hand, and standing litter of *S. scoparium* was harvested at a height of 5 cm from the soil surface to avoid soil contamination and minimize exposure to soil decomposers. Once collected, I immediately dried the litter at 65 °C until constant mass was reached. I used 6 g of litter from each species from each plot to determine initial litter quality, and used the remaining litter to construct litter bags.

I constructed 20 × 20 cm litter bags using 1-mm fiberglass mesh, with each bag containing 4 g of litter. Because I expected increased rainfall variability and N addition to influence litter decomposition and N loss

directly through changes in the decomposition environment, and also indirectly through changes in litter quality, I filled enough bags to allow me to decompose litter from each plot in that same plot, and also in each of the other three plots within that block. Below, I refer to the effects of conditions during litter formation (i.e., the 2012 growing season) as origin effects (OV: origin variability, ON: origin N) and the effects of conditions during decomposition (i.e., 2013) as destination effects (DV: destination variability, DN: destination N). I took litter bags from each origin condition (full control, increased rainfall variability, added nitrogen, or increased rainfall variability and added nitrogen) and decomposed them within each destination condition (full control, increased rainfall variability, added nitrogen, or increased rainfall variability and added nitrogen). I filled 288 litter bags, enough to accommodate three collection dates (2 species × 4 origin conditions × 4 destination conditions × 3 collection dates × 3 replicates).

Litter bags were deployed in December 2012. They were randomly placed along either the eastern or the western perimeter of each plot and spaced at least 20 cm apart and 1 m from the plot's exterior edge. Litter bags were pinned to the soil surface by bamboo skewers and collected after either 60, 210, or 365 days. These collection dates were selected in response to the timing of the PRICLE experimental manipulations. The first collection took place after 60 days of field incubation (February 2013), when only the indirect effects of the treatments (e.g., changes in litter quality or legacy effects on the decomposer community) would have influenced litter, since the experimental manipulations had been inactive. Thus, any destination effects of increased variability or N addition at this time point could only have been attributed to legacy effects from the season before. The second collection date, 210 days (July 2013) took place approximately halfway through the 2013 experimental period and 28 days after the 2013 N addition. The third and final collection date, 365 days (December 2013), took place two full months after the final watering event of the growing season and provided an integrative measure of direct and indirect effects after a year of field incubation. One litter bag of each species was lost to animal activity and was not collected at 365 days. After collection, litter was dried at 65 °C and cleaned by gently brushing to remove soil, rocks, and other contaminants. Once cleaned, litter was weighed, ground through a Wiley mill, and then pulverized in a ball mill. I followed the same grinding protocol for the 6 g of undecomposed litter from each plot set aside at the beginning of the experiment. Subsamples of all pulverized material, and of mineral soil samples collected from each plot, were analyzed for C and N concentration using an ECS 4010 element analyzer (Costech Analytical Technologies, Valencia, CA, USA).

Statistical analyses

To evaluate the impacts of increased rainfall variability and N addition on litter quality, I conducted a mixed model analysis of variance (ANOVA) considering initial litter C:N from each species from each plot as a response variable and origin variability, origin N, and species as fixed categorical factors with two levels each. I also included block as a random categorical factor.

In order to assess the influences of increased rainfall variability and N addition on litter decay, I performed a series of mixed model ANOVAs considering origin and destination effects on mass and N loss. Using the C correction method (Robertson 1999) to account for soil contamination, I calculated mass and N loss for each litter bag as a percent of initial mass (g) of litter and N, respectively. Thus, mass and N losses are defined as the amount of litter or N lost as a percent of the total initial mass of litter or N, respectively. Mass loss was square-root-transformed to meet the assumptions of normality. These analyses considered either N loss or square-root-transformed mass loss as response variables and origin variability, origin N, destination variability, destination N, and time as categorical factors. I allowed interactions between origin effects, destination effects, and time. Block was included as a random factor. Changes in mass loss and N loss (as discussed throughout the manuscript) were calculated using least squares means and are expressed as percent deviation from the least squares mean of the reference condition for the considered factor (e.g., if N loss increases from 50 % under ambient rainfall variability to 75 % under increased rainfall variability, I refer to this as a 50 % increase in N loss).

I also examined whether initial litter C:N exerted stronger or weaker control over litter decay under increased rainfall variability or N addition. To do this, I used mixed models to analyze the response of square-root-transformed mass loss or N loss (as percentages) to destination conditions and time (categorical factors) and initial litter C:N (continuous factor). Block was included as a random factor. By including interactions of destination conditions, time and initial litter C:N, I was also able to examine how the sensitivity of mass and N loss to litter C:N ("substrate sensitivity") changed in response to increased rainfall variability and N addition. In cases where destination variability, destination N, or time significantly interacted with initial litter C:N, I performed linear regressions of mass loss or N loss (as percentages) by initial litter C:N (g C g N^{-1}) for each treatment
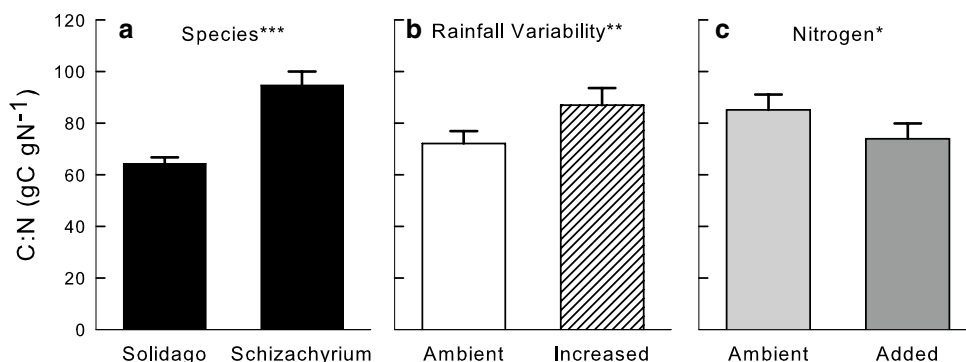
combination in the interaction and defined substrate sensitivity as the slope coefficient of the C:N parameter. These analyses were conducted separately for each species in order to avoid issues of colinearity between species and C:N ratio.

All statistics were performed in SAS v.9.3 (SAS Institute) using PROC MIXED for all mixed models.

Results

Origin effects on litter C:N

Initial litter C:N varied greatly by species and was influenced strongly by both rainfall variability and N treatments, resulting in a large range of initial litter chemistry values (Fig. 1). Across all plots, *S. canadensis* litter had a lower initial C:N (61 ± 2 g C g N^{-1}; mean ± SE) than that of *S. scoparium* (90 ± 5 g C g N^{-1}; Fig. 1a). Litter from increased rainfall variability plots had 21 % higher C:N than litter from ambient rainfall variability plots ($F_{1,14} = 11.79$, $p = 0.004$; Fig. 1b) and litter from N addition plots had a 13 % lower C:N than litter from ambient N plots ($F_{1,14} = 6.69$, $p = 0.022$; Fig. 1c). Neither the effects of increased rainfall variability ($F_{1,14} = 2.76$, $p = 0.119$) or N addition ($F_{1,14} = 0.20$, $p = 0.903$) on litter C:N varied by species.

Origin effects on *S. scoparium* mass and nitrogen loss

Schizachyrium scoparium litter retained a majority of its mass over the experimental period and continued to immobilize N leading up to the day 365 collection (Fig. 2a, c). Litter from N-addition plots lost mass more quickly than that from ambient N plots ($F_{1,93} = 4.79$, $p = 0.031$), but only if the tissue had also grown under increased rainfall variability ($F_{1,93} = 4.50$, $p = 0.037$). Origin conditions affected N loss more strongly than mass loss. Litter from plots subjected to increased variability immobilized 147 % more N than litter from ambient rainfall plots ($F_{1,93} = 17.16$, $p < 0.0001$).
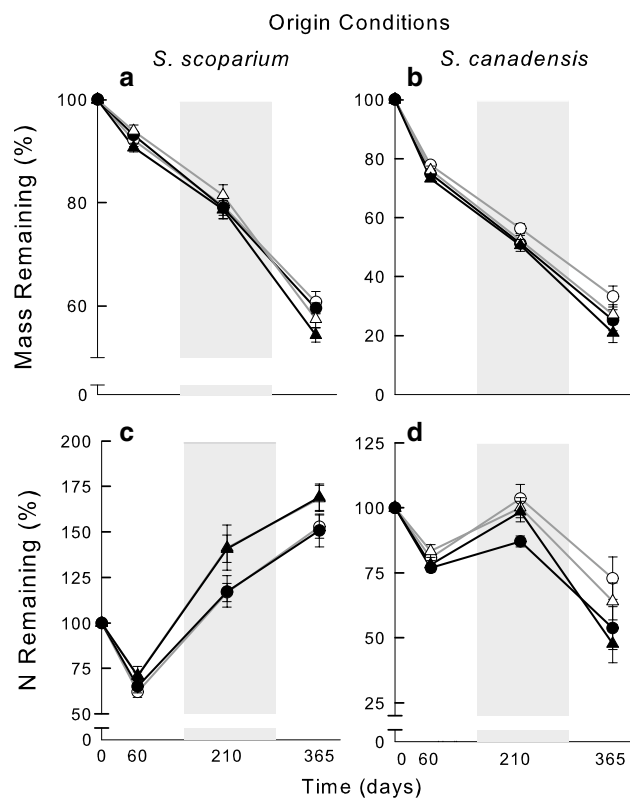

Fig. 2 Mean (±SE) mass (**a**, **b**) and N (**c**, **d**) remaining for *S. scoparium* (**a**, **b**) and *S. canadensis* (**b**, **c**) litter bags based on origin rainfall variability (ambient: *circle*, increased: *triangle*) and origin N availability (ambient N: *open symbols with gray lines*, N addition: *closed symbols with black lines*). *Shading in each panel* corresponds to the growing season and duration of the experimental manipulations. $n = 3$ for all except N addition + increased variability *S. scoparium* and *S. canadensis* at 365 days, for which $n = 2$

Origin effects on *S. canadensis* mass and nitrogen loss

Solidago canadensis litter decomposed more quickly than that of *S. scoparium*, and exhibited net N immobilization only briefly (depending on origin and destination conditions) before releasing N for the remainder of the experimental period (Fig. 2b, d). Origin conditions affected both

mass and N loss of *S. canadensis,* with increased variability ($F_{1,93} = 9.10$, $p = 0.003$) and added N ($F_{1,93} = 20.57$, $p < 0.0001$) enhancing mass loss by 6 and 10 % over the course of the experiment compared to ambient variability and ambient N conditions, respectively. Overall N loss from N-addition litter was also 65 % greater than that of ambient N litter ($F_{1,93} = 18.25$, $p < 0.0001$).

Destination effects on *S. scoparium* mass and nitrogen loss

Environmental conditions during litter decomposition had only minor influences on mass loss of *S. scoparium* litter (Fig. 3a). Increased rainfall variability had no significant effect on *S. scoparium* mass loss, and N addition only stimulated mass loss at 365 days ($F_{2,93} = 3.74$, $p = 0.028$).

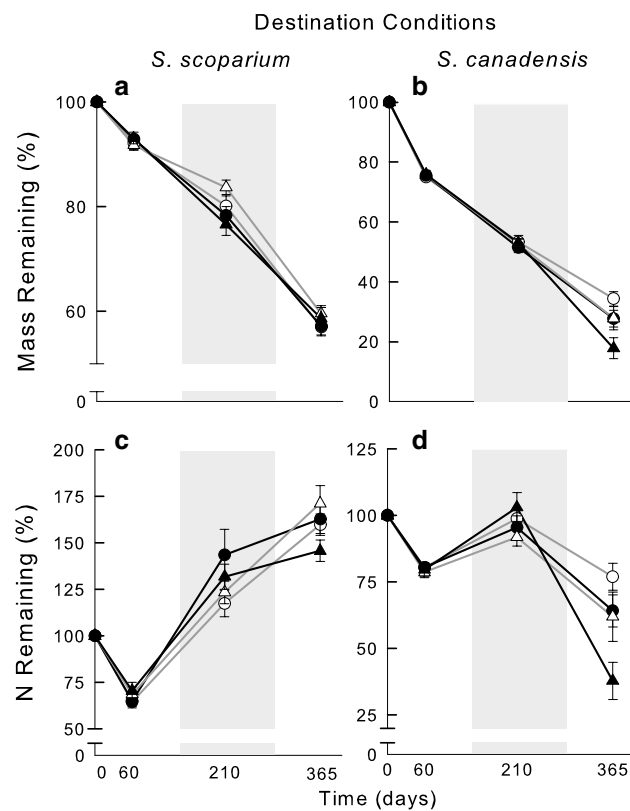Nitrogen release of *S. scoparium* litter was influenced by both N addition and increased rainfall variability during decomposition (Fig. 3c). Effects of destination N conditions were time-dependent. Compared to litter decomposed under ambient N, additional N reduced N immobilization in *S. scoparium* litter at 365 days by 16 %, but it also increased N immobilization at 210 days by 84 % ($F_{2,93} = 4.67$, $p = 0.012$), approximately 30 days after the N fertilizer was applied. When applied in combination with increased rainfall variability, N addition also showed a trend of reducing N immobilization at 365 days more strongly than N addition alone ($F_{1,93} = 3.81$, $p = 0.054$). Rainfall variability and N addition during litter decomposition also interacted with origin conditions (OV × ON × DV × DN) to slow N release from *S. scoparium* litter that originated from increased rainfall variability, ambient N plots ($F_{1,93} = 4.44$, $p = 0.038$; Online Resource 2).

Destination effects on *S. canadensis* mass and nitrogen loss

Destination conditions were more influential for *S. canadensis* litter mass loss than for that of *S. scoparium* litter (Fig. 3a, b). Adding N increased overall mass loss of *S. canadensis* litter ($F_{1,93} = 5.36$, $p = 0.023$), but this effect was largely the result of increased mass loss at 365 days ($F_{2,93} = 4.81$, $p = 0.010$). Increased rainfall variability also increased mass loss of *S. canadensis* litter, but this effect was only detectable after 365 days ($F_{2,93} = 5.10$, $p = 0.008$). Furthermore, destination variability, time, and origin nitrogen interacted, such that increased rainfall variability during decomposition and N addition during plant growth acted synergistically to enhance mass loss, but only at 365 days ($F_{2,93} = 4.71$, $p = 0.011$; Online Resource 2).

Nitrogen loss from *S. canadensis* litter was the most responsive process I measured to increased rainfall variability and N addition during litter decay (Fig. 3d). Adding N increased N loss of *S. canadensis* by 57 % over the course of the experiment ($F_{2,930} = 7.74$, $p = 0.001$). Increased rainfall variability also increased N loss of *S. canadensis* litter ($F_{1,93} = 8.31$, $p = 0.005$), but this was largely driven by litter collected at 365 days ($F_{2,93} = 7.18$, $p = 0.001$), where N loss was enhanced by 66 %. Together, added N and increased rainfall variability showed a marginally significant ($F_{2,93} = 2.65$, $p = 0.076$) trend to synergistically increase N loss at 365 days by 156 % over fully ambient conditions. In a two-way interaction between destination variability and origin N, *S. canadensis* litter from N addition plots responded more strongly to increased rainfall variability: in litter from these plots, N loss was enhanced by 58 %, compared to 14 % for litter from ambient N plots ($F_{1,93} = 4.06$, $p = 0.047$, Online Resource 2).

Fig. 3 Mean (±SE) mass (**a**, **b**) and N (**c**, **d**) remaining for *S. scoparium* (**a**, **c**) and *S. canadensis* (**b**, **d**) litter bags based on destination rainfall variability (ambient: *circle*, increased: *triangle*) and destination N availability (ambient N: *open symbols with gray lines*, N addition: *closed symbols with black lines*). *Shading in each panel* corresponds to the growing season and duration of the experimental manipulations. *n* = 3 for all except N addition + increased variability *S. scoparium* and ambient N + ambient variability *S. canadensis* at 365 days, for which *n* = 2

Substrate sensitivity of *S. canadensis* and *S. scoparium* litter decay

Over the entirety of the experiment, litter C:N was a relatively poor predictor of mass loss. Mass loss of *S. scoparium* was not strongly controlled by initial litter C:N regardless of destination conditions ($F_{1,119} = 1.62$, $p = 0.206$), and was therefore relatively insensitive to substrate quality. However, litter quality of *S. canadensis* was a marginally significant predictor of future mass loss ($F_{1,117} = 3.77$, $p = 0.055$) and the substrate sensitivity of mass loss— defined as the slope of the relationship between initial litter C:N (g C g N^{-1}) and percent mass loss (0.01 g g^{-1})—for *S. canadensis* was greater under increased rainfall variability (Online Resource 3). *S. canadensis* litter with lower C:N lost relatively more mass under increased rainfall variability, particularly after 365 days of decomposition ($F_{2,117} = 6.67$, $p = 0.002$).

Nitrogen loss was more sensitive to litter quality and litters with lower C:N ratios lost a greater proportion of N than litters with higher C:N ratios for both species (*S. scoparium*: $F_{1,119} = 73.70$, $p < 0.0001$; *S. canadensis*: $F_{1,117} = 17.49$, $p < 0.0001$). The substrate sensitivity of N loss—defined as the slope of the relationship between initial litter C:N (g C g N^{-1}) and percent N loss (0.01 g N g N^{-1})—was roughly equivalent between species (-8.5×10^{-3} g N g C^{-1} for *S. canadensis* and -8.7×10^{-3} g N g C^{-1} for *S. scoparium*). Despite this, the substrate sensitivity of N loss for *S. scoparium* varied more across the treatments than that of *S. canadensis* (Fig. 4). *S. canadensis* substrate sensitivity increased under increased rainfall variability ($F_{1,117} = 10.93$, $p = 0.001$), exacerbating the difference between higher- and lower- C:N litters' N loss, but only at 365 days ($F_{2,117} = 8.12$, $p = 0.001$; Fig. 4d). In contrast, increased rainfall variability reduced the substrate sensitivity of N loss for *S. scoparium* litter, but only at 210 days ($F_{2,119} = 3.91$, $p = 0.023$; Fig. 4c). Additionally, substrate sensitivity of N loss for *S. scoparium* increased under N addition at 210 days ($F_{2,119} = 5.83$, $p = 0.004$; Fig. 4a), when higher C:N litter immobilized even more of the added N compared to lower C:N litter than it would have otherwise. This effect was offset by increased rainfall variability ($F_{1,119} = 4.95$, $p = 0.028$).

Complete statistical results from each model can be found in Online Resource 4

Discussion

Increased rainfall variability and N addition during litter formation and decomposition had distinct influences on litter decay, but the strength of these effects varied by species and over time. Both treatments almost always increased
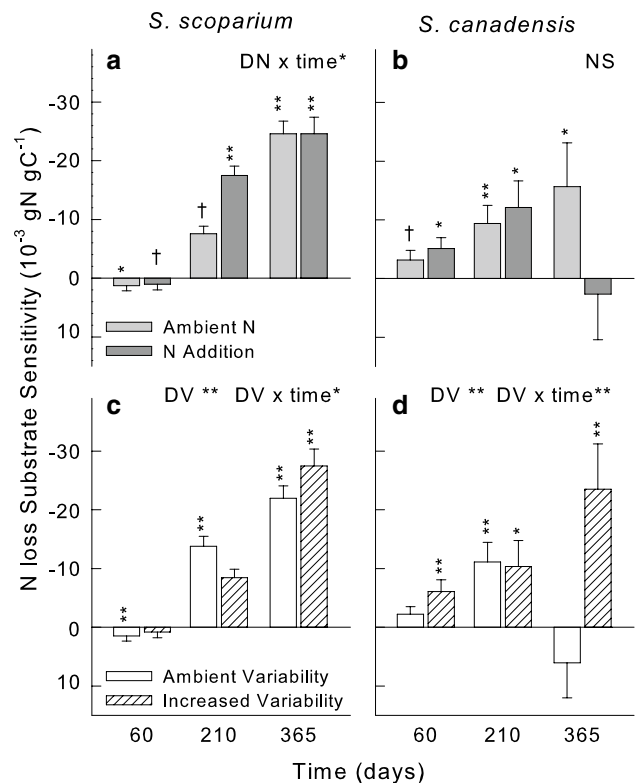

Fig. 4 Sensitivity of *S. scoparium* (**a**, **c**) and *S. canadensis* (**b**, **d**) N loss to initial litter C:N ratio. Values are slope estimates resulting from regressions of N loss (as a percent of initial g N) by initial C:N (gC gN^{-1}) on pooled data across litter bags exposed to N (**a**, **b**) and rainfall variability (**c**, **d**) treatments during decomposition. Ambient rainfall (*open bars* in **c**, **d**), increased rainfall variability (*hashed bars* in **c**, **d**), ambient N (*light shaded bars* in **a**, **b**), and N addition (*dark shaded bars* in **a**, **b**) during decomposition. Values significantly different from zero are marked. $^{\dagger}p \leq 0.10$, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ ($n = 24$ for all except added N and increased rainfall variability *S. scoparium* at 365 days, and ambient N and ambient rainfall variability *S. canadensis* at 365 days, for which $n = 23$)

nutrient cycling rates in litter of *S. canadensis*, despite changes in litter quality that would suggest otherwise. In contrast, *S. scoparium* litter was more resistant to environmental conditions, and maintained relatively slow rates of mass and N loss.

Origin conditions

In agreement with previous studies, plants grown under N addition (Valera-Burgos et al. 2013) or increased rainfall variability (Fay et al. 2002) produced litter with lower or higher C:N, respectively. However, I found that increased rainfall variability had a larger effect on litter quality than N addition, which is intuitive considering that changes in precipitation patterns affect the availability of multiple soil resources, including water and nutrients, as opposed to only N (Everard et al. 2010), and also alter plant productivity

and internal nutrient cycling (Knapp et al. 1998). These two treatments had effects of equal magnitude but oppose direction on litter N concentration, suggesting that 5 g N m^{-2} (roughly triple ambient N deposition; National Atmospheric Deposition Program) has as much influence on within-plant N cycling as the tenfold increase in heavy rainfall events applied to the increased variability treatment. The effects of anthropogenic N deposition on ecosystem function and biodiversity loss have been well studied (Vitousek et al. 1997; Sala et al. 2000; Bobbink et al. 2010), but ecosystem responses to variation in rainfall patterns associated with climate change have received significantly less attention (Kreyling and Beier 2013). The finding that increased rainfall variability could influence litter dynamics to a greater extent than N addition adds to the growing body of literature evaluating ecosystem responses to rainfall variability (e.g., Kulmatiski and Beard 2013; Fry et al. 2014) and suggests that more variable precipitation regimes can affect ecosystem processes and deserve more attention.

S. canadensis litter derived from increased rainfall variability plots decomposed faster than litter from ambient rainfall plots, despite having a higher C:N. At the same time, origin rainfall had no detectable influence on decomposition of *S. scoparium* other than to increase sensitivity to N addition during decomposition, even though litter from increased rainfall variability plots had a higher C:N that would have led me to expect a slower decomposition rate. These unexpected results suggest that increased rainfall variability likely altered some leaf traits in ways not captured by C:N, and that these changes favored faster decomposition enough to override any effects linked to C:N. A pilot study conducted at the same site in 2013 found that under more variable rainfall, *S. canadensis* produced less dense leaves (higher specific leaf area; Online Resource 5), which can hold more water and provide more favorable microclimates for microbial decomposers than more dense leaves (Makkonen et al. 2013). Although I did not measure the water-holding capacity of litter produced in 2012, I expect that this litter trait is at least partially responsible for the counterintuitive result of increased rainfall variability producing higher C:N litter that also decomposed more quickly.

Destination conditions

Destination conditions strongly regulated litter mass and N loss. I observed no differences in mass loss or N loss between litter bags based on destination until the 210-day collection, suggesting that any legacy effects of treatments during the 2012 season on decomposing environment in 2013 were not a major determinant of litter decay during the first 60 days of decomposition. N addition initially reduced N loss and increased N immobilization in litter of both species, which makes sense considering that plots were fertilized only 28 days before the collection date, and indicates that the supplemental N stimulated the microbial community. However, this initial increase in litter N is also likely responsible for fueling microbial activity and increasing N loss by 365 days (Janssen 1996).

The combination of N addition and increased rainfall variability influenced the decomposition of *S. canadensis* and *S. scoparium* differently. For *S. canadensis,* losses of mass and N from litter were generally enhanced by increased rainfall variability, and there was a trend of this effect being stronger under N addition. This was consistent with my hypotheses, since I expected N to favor the breakdown of labile substrates and because higher soil moisture content, as was observed with increased rainfall variability in 2013, typically promotes faster decomposition and N release under mesic conditions (Dyer et al. 1990). In contrast, N additions reduced immobilization in *S. scoparium* litter, but only under ambient rainfall conditions, indicating that increased rainfall variability somehow negated this effect and stimulated N immobilization. These trends were also reflected within species, where increased rainfall variability increased substrate sensitivity of N loss from *S. canadensis,* but reduced substrate sensitivity of N loss from *Schizchyrium* (Fig. 4c, d). One possible explanation is that the large rainfall events during decomposition evoked litter-specific responses from the microbial communities associated with each type of litter, promoting N immobilization in the high C:N litter of *S. scoparium* and promoting net N mineralization in the low C:N litter of *S. canadensis* (Appel 1998; Austin et al. 2004). However, I did not measure microbial activity directly, and therefore I can only speculate as to the cause of this trend.

I had anticipated interactions between origin and destination conditions, but found only a two-way interaction for *S. canadensis* N loss (ON × DV), a three-way interaction for *S. canadensis* mass loss (ON × DV × Time), and a four-way interaction for *S. scoparium* N loss (OV × ON × DV × DN). While I had expected that changes in litter chemical and physical properties brought on by increased rainfall variability and N addition would result in a greater spread of litter susceptibility to those same global change factors later on in the experiment, these three interactions led me to two conclusions. First, the relatively minor origin–destination effects on litter mass loss compared to the larger origin–destination interaction for N loss of *S. canadensis* suggest that litter N dynamics may be more susceptible to concurrent changes in environmental conditions than litter mass loss. Second, the origin–destination interactions I detected corroborate my findings of altered substrate sensitivity and suggest litter C:N as a mechanism driving these interactions. Indeed, *S. canadensis* litter from

N addition plots, which had a lower C:N ratio, lost more N under increased rainfall variability. Similarly, *S. scoparium* litter grown under increased rainfall variability or ambient N conditions, which had higher C:N, immobilized more N under either increased rainfall variability or N addition compared to lower C:N litters. These results suggest that increased rainfall variability and N addition will exacerbate differences in N release between litters of differing C:N in the future. Differences in litter C:N are likely to become greater over several years as smaller effects of global change accumulate to influence plant nutrient use (Seastedt et al. 1991; Knapp et al. 1998), thus making changes in substrate sensitivity more important over time.

Implications for system C and N cycling

While both origin and destination conditions influenced decomposition and N loss, the relative importance of the treatments during litter formation versus decomposition varied between species. Origin conditions were more important for *S. scoparium* litter dynamics than destination conditions, likely as a result of the species' poor litter quality. The high C:N ratio of *S. scoparium* litter requires microbial decomposers to supplement the existing litter with exogenous N (Melillo et al. 1982; Hobbie and Vitousek 2000). Thus, litter quality is limiting to both mass and N loss and restricts the ability of external factors to expedite the decomposition process. So, while destination conditions were able to moderate mass and N loss, litter traits influenced by origin conditions were primarily responsible for the observed rates of decay in *S. scoparium.* In contrast to *S. scoparium*, *S. canadensis* litter had a much lower C:N ratio and, even though origin conditions likely modified multiple litter traits, those changes in litter quality were less important because microbial activity was limited more by external (destination) conditions. Thus, destination conditions had a larger influence on decay of *S. canadensis* than *S. scoparium*. Although the experiment had only been running for a year before the litter bag trial began, these results imply varying degrees of legacy effects from plant growing conditions on ecosystem C and N cycling due to the litter quality of each species, and provide important insight into the mechanisms mediating the response of litter decomposition to global change. In prairie communities dominated by *S. scoparium* or other high C:N litter species, these processes may be more sensitive to the previous year's climatic and nutrient conditions, while in communities dominated by species with low C:N litter, like *S. canadensis,* conditions during decomposition have relatively stronger effects.

I found that increased rainfall variability and N addition stimulate decomposition and N release. These results indicate that potential future changes in N deposition and rainfall variability could generally accelerate C and N cycling, particularly in *S. canadensis*-dominated communities; although changes in other conditions, such as atmospheric CO_2 and temperature, will also play strong roles. Smith et al. (unpublished) found that N addition and increased rainfall variability in this experiment favored the expansion of *S. canadensis*, and pushed communities to be heavily dominated by this clonal forb. Thus, with the combination of greater abundance of *S. canadensis* and enhanced decay rates of its litter, element cycling rates in the system will likely accelerate as N deposition and rainfall variability increase, potentially increasing nutrient availability. Increased fertility through this mechanism could contribute to a previously unknown positive feedback where increased rainfall variability and N deposition favor *S. canadensis*, enhance decomposition rates and soil N availability, and thin litter layers, which in turn favor even greater *S. canadensis* abundance and thinner litter layers (Knapp et al. 1998). However, since the differences between *S. canadensis* and *S. scoparium* litter quality and decay far exceeded differences induced by increases in rainfall variability and N deposition, shifts in plant community composition are likely to play a larger role in determining responses of nutrient cycling to projected increases in either global change factor. The findings of this study and of others at the same experimental site lead me to suggest that projected increases in rainfall variability and anthropogenic N deposition will accelerate nutrient cycling rates in tallgrass prairie, and that this could feed back to further influence soil C pools and plant community composition.

Author contribution statement MJS designed the experiment, conducted the field work, performed all analyses, and prepared the manuscript.

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