

Ecological impacts of the invasive grass *Sorghum halepense* on native tallgrass prairie

Marnie E. Rout · Thomas H. Chrzanowski ·
William K. Smith · Laura Gough

Received: 30 November 2010 / Accepted: 23 July 2012
© Springer Science+Business Media B.V. (outside the USA) 2012

Abstract Invasive plants frequently have competitive advantages over native species. These advantages have been characterized in systems in which the invading species has already become well established. Surprisingly, invader impacts on native communities *currently undergoing* invasion are lacking from most ecological studies. In this work we document and quantify shifting patterns in plant community structure in a native ecosystem (remnant tallgrass prairie) undergoing invasion by the invasive exotic *Sorghum halepense* (Johnsongrass). Further, we use manipulative field and greenhouse studies to quantify impacts of potential allelochemicals contained in whole-plant *S. halepense* leachates on growth of the dominant native grass, *Schizachyrium scoparium* (Little Bluestem), and tested the inhibitory effects of the potential soil legacy of *S. halepense* on the native grass in the greenhouse. Plant diversity indices revealed three

distinct plant communities within the remnant prairie: a native community, a densely *S. halepense* invaded area, and a transitional zone between the two. Dominance of the native grass, determined by relative percent cover, significantly declined with increased *S. halepense* invasion via rhizomatous growth. Annual global positioning system monitoring of the *S. halepense* invasion front was used to quantify advancement into native prairie, documented at an average rate of 0.45 m year^{-1} . In the manipulative field and greenhouse studies, native *S. scoparium* treated with invasive *S. halepense* leachate had significantly less biomass and fewer inflorescences than control plants. These findings indicate the prolific clonal growth in conjunction with the plant chemistry of *S. halepense* play a significant role in displacement of the native grass.

Keywords Clonal growth · Ecological impact · Invasion ecology · Leachate · *Schizachyrium scoparium* · *Sorghum halepense*

M. E. Rout · T. H. Chrzanowski · L. Gough
Department of Biology, The University of Texas
at Arlington, Arlington, TX 76019, USA

Present Address:
M. E. Rout (✉)
Fort Keogh Livestock & Range Research Laboratory,
USDA-ARS, Miles City, MT 59301, USA
e-mail: marnie.rout@mso.umt.edu;
marnie.rout@ars.usda.gov

W. K. Smith
Numerical Terradynamic Simulation Group,
The University of Montana, Missoula, MT 59812, USA

Introduction

Invasive plants have garnered much attention in recent years. They pose serious economic costs as weeds (Vitousek et al. 1997; Pimentel et al. 2000), generally reduce native species diversity (Wilcove et al. 1998), and can alter broad scale ecosystem functioning

(D'Antonio and Vitousek 1992; Gordon 1998; Levine et al. 2003). Alterations to ecosystem functions in the context of plant invasions have gained considerable interest in recent years, due in part to documented impacts on soil biogeochemical cycles (Liao et al. 2008) and what these changes imply about interactions among invasive plants and broad functional roles of microbes involved in nutrient dynamics, particularly nitrogen cycling (Rout and Callaway 2009; Rout and Callaway 2012). Considerable effort has focused on factors regulating species establishment and persistence in non-native ecosystems, spanning factors from genetic composition and regulation of gene expression to plant life-history strategy. For example, Strauss et al. (2006) showed that relatedness to native populations is one factor contributing to invasive plant success (i.e., the more novel the genera, the more successful an invader), while Gough et al. (2012) demonstrated that a life-history strategy of clonal fragmentation coupled with canopy dominance (height advantage) conferred a competitive advantage in North American herbaceous plant communities, irrespective of plant status in the community (invasive, native, naturalized). The invasive plant of interest in this work, *Sorghum halepense*, fits both of these criterion.

Plants of the *Sorghum* genus are novel to the North American continent, including the globally successful invader *S. halepense* (Johnsongrass), a hybrid between *S. bicolor* and *S. propinquum*, proposed to be of Mediterranean origin (Paterson et al. 1995). Genes regulating rhizome growth in *S. halepense* are derived primarily from *S. propinquum* (Jang et al. 2006), although this progenitor species is not nearly as prolific at rhizome production. In fact, extensive rhizome production is well documented in this species (Knopf and Scott 1990; McWhorter 1961, 1981), a trait in common with many of the world's most aggressive plant invaders (Pyšek 1997), and plants utilizing this clonal growth form have been shown to invade, persist, and dominate regardless of soil conditions (Prach and Pyšek 1994). In addition to its genetic novelty in North America and prolific clonal rhizomatous growth, the plant has several other properties that lend to its invasive character. As a robust seed producer, it is capable of self-fertilization generating upwards of 100,000 seeds plant⁻¹ (Knopf and Scott 1990). Chemically, it produces the constitutive defense compound dhurrin in the leaves (a cyanogenic glycoside, Nielsen and Moller 1999) and the allelopathic molecule, sorgoleone, is

exuded from root hairs (Czarnota et al. 2001; Kagan et al. 2003). Recently, *S. halepense* was found to harbor N₂-fixing bacterial endophytes in the rhizomes and dense monocultures were correlated with increased concentrations of several plant-essential nutrients in soils (Rout and Chrzanowski 2009).

The annual cycle of *S. halepense* clonal growth and senescence is relevant to the plant's defense and allelochemistry. Growth of rhizomes continues throughout the year and may reach as high as >60 m year⁻¹ plant⁻¹ (McWhorter 1961, 1981). Ramets erupt in early spring, primarily from buds on rhizomes, contributing to dense clonal monocultures. The allelopathic chemical sorgoleone and its derivatives are continually exuded from root hairs (Czarnota et al. 2003). When the plant is crushed, cyanide in the dhurrin (contained in leaves) is released making it toxic to herbivores (Nielsen and Moller 1999), thus consumption of plant material is minimal, from both living material and senesced litter. Plant senescence occurs upon exposure to freezing temperatures, at which point culms (>2 m height) fall, creating a thick, dense mat of litter (McWhorter 1981). In portions of the US that receive significant rain during the fall and winter months, water-soluble chemicals and nutrients contained within the plant can effectively leach into the surrounding soils, while others have speculated that water-insoluble phenolics that act as allelochemicals can be released from the plant through decomposition (Weston et al. 1989).

The mode of action of dhurrin is through cyanide toxicity (Halkier and Moller 1989). By inhibiting mitochondrial cytochrome oxidase, cyanide blocks electron transport and oxygen metabolism is severely hindered at the cellular level. Several break-down products of dhurrin have also been identified as phytotoxic, including the phenolic compound *p*-hydroxy benzoic acid (Weston et al. 1989). Similar to the mode of action of dhurrin, the allelopathic compound (sorgoleone) also targets respiratory pathways (Nimbal et al. 1996). More specifically, sorgoleone inhibits photosynthesis (Gonzalez et al. 1997; Rimando et al. 1998), enzyme synthesis (Meazza et al. 2002), mitochondrial respiration (Czarnota et al. 2001), and solute and nutrient uptake (Hejl and Koster 2004) in plants. In addition, sorgoleone has been shown to persist for months in soils (Czarnota et al. 2003; Weidenhamer et al. 2009).

Interestingly, the impacts of clonal growth combined with height advantage have been shown to

maximize competitive ability at high soil fertility levels (Wilson and Tilman 1993; Gough et al. 2012), similar to levels previously documented in densely invaded *S. halepense* soils (Rout and Chrzanowski 2009). In general, there is a distinct lack of information for many exotic plant species (including *S. halepense*) characterizing the relationship between their chemical properties, their growth forms, and successful invasion and persistence in the context of native landscapes (Hierro and Callaway 2003; Inderjit et al. 2001; Inderjit and Callaway 2003).

In this study, we address the role of clonal growth in conjunction with plant chemistry in the competitive success of *S. halepense* (hereafter *Sorghum*) in a natural community (tallgrass prairie) experiencing invasion by using material leached from whole plants (hereafter leachates). Through the application of water-based leachates, we addressed the potential for water-soluble extracts from *Sorghum* tissues to act as allelopathic inhibitors on the dominant native grass (*Schizachyrium scoparium*, Little Bluestem, hereafter *Schizachyrium*), in field and greenhouse experiments. Additionally, we addressed the impact of the potential legacy of this invasive grass in densely invaded soils on the native grass in a greenhouse experiment. We report the results of these manipulative experiments on growth of vegetative and reproductive structures of the dominant native grass. We hypothesized that (1) leachates made from live tissues of *Sorghum* would negatively affect growth of the native grass; (2) *Sorghum* invaded soils would inhibit growth of the native grass; and (3) treatments where the native grass was growing in invaded soils and receiving leachate would be the most negatively affected.

Methods

Study site description and history

The approximately 12 ha study site was located at the convergence of the Fort Worth Prairie and Western Crosstimbers ecoregions inside the Fort Worth Nature Center and Refuge (FWNC&R) (32° 84' N, 97° 47' W, ~200 m elevation), Fort Worth, TX. Fort Worth Prairie is characterized by a shallow gravel alkaline clay-based soil underlain by limestone bedrock (Diggs et al. 1999). The dominant vegetation (based on percent cover) is *Schizachyrium*, while *Bouteloua*

curtipendula (Sideoats Grama), *Sporobolus compositus* (Tall Dropseed), *Bouteloua hirsuta* (Hairy Grama), *Sorghastrum nutans* (Indiangrass), and *Andropogon gerardii* (Big Bluestem) comprise the other common native grasses characteristic of Fort Worth Prairie (Diggs et al. 1999).

Over four decades ago, the study area was split approximately in half where an area in the northern portion was used for a community garden plot, while the southern portion remained as undisturbed prairie. Since acquisition by the City of Fort Worth in 1967, the area has not been used for community gardens and has been irregularly burned and mowed (S. Tuttle, Director FWNC&R, personal communication). The northern section is undergoing extensive invasion by *Sorghum* (hereafter, invaded prairie) that is extending into the remnant native prairie. *Sorghum* is advancing as a distinct invasion-wave or front that is visually distinguished by a shift in the vegetation. The invasion-front creates a transition zone between invaded and native prairie characterized by *Sorghum* emergence from rhizomes in areas where the plant was not previously found, thus extending the leading edge of the invasion into the native prairie.

Target species

Schizachyrium scoparium, is a C₄ perennial caespitose grass and was chosen as the focal species due to its historical dominance among vegetation of the tallgrass prairie ecosystem, specifically Fort Worth Prairie (Diggs et al. 1999). It relies on both clonal fragmentation and sexual reproduction through seeds for population maintenance and dispersal. More specifically, tiller hierarchies are created by growth of daughter tillers within recalcitrant crowns (hereafter crown base), and fragmentation of this results in the dispersal of clones (clonal fragmentation, Butler and Briske 1988).

Monitoring invasion progression and defining community structure

The *Sorghum* invasion line was routinely mapped during the height of the growing season (July) over the course of the study period (2004-2009) using global positioning system (GPS) techniques (Trimble GSX, Sunnyvale, CA) to quantify the rate of invasion. Initially in July 2004, GPS coordinates were recorded for 37 approximately equal spaced points (~2 m

apart) along the invasion front, establishing 37 longitudinal transects. In subsequent years, shifts along the invasion front were monitored by recording the advance or retreat of *Sorghum* along each of the 37 points (latitude was held constant while longitudinal invasion front changes were recorded). Only changes along the primary direction of the invasion (i.e. longitudinal changes) were monitored to minimize measurement error. Net invasion progression was determined by point-to-point comparison among transects by calculating annual longitudinal differences, converted into meters using the Cosine-Haversine formula (Robusto 1957), where at a reference latitude of 32.83°, 1 degree of longitude was calculated to be 93,430 m. The Cosine-Haversine formula is based on a spherical Earth; however, given the relatively small magnitude of invasion change, error introduced due to ellipsoidal effects were negligible.

Community structure was determined by establishing 36 1-m² plots in July 2005 to create four parallel transects running approximately perpendicular to the invasion front (north–south). The invasion line bisected all four transects. Each transect was 150 m long, contained 9 1-m² plots spaced 15 m apart, and was separated from the adjacent transect by 10 m. Plant community-types for each plot within each transect were defined based on percent relative aerial cover of all vascular plant species, assessed annually in July 2005–2007.

Manipulative experiments

Invasive plant leachate

A monoculture of *Sorghum* inside the FWNC&R but separate from the experimental area served as a source of plants for creation of a leachate that would be subsequently applied to *Schizachyrium*. Given the nature of *Sorghum* growth and senescence (see above), we chose to create a leachate from whole plants throughout the growing season. Plants were harvested from 20 plots (~25 × 25 cm) weekly during the duration of the experiment (23 weeks, Apr.–Aug. 2004) and immediately transported to lab facilities. Living plant material (rhizomes, roots, shoots and leaves) was gently rinsed in deionized, carbon-filtered, reverse osmosis (DICRO) water, cut into ~ 5 cm segments, and weighed. Equal amounts (700–900 g) were placed in four Nitex bags (80 µm mesh-size),

each submerged in 16 L of DICRO water and leached for 24 h. Mixing was achieved by aeration. The leachate (64 L) was pooled, filtered through course-mesh cheesecloth, and finally filtered through Whatman filters (10 µm-porosity, 144 mm-diameter). Approximately 500 mL of each batch of leachate was frozen (–20 °C) for later chemical analyses, and the remainder stored at 4 °C until application in the field and greenhouse, which occurred within 24 h for 17 of the 18 applications, and within 7 days for the remaining one due to rain events delaying treatment.

Leachate phosphorus (P) content was determined from persulfate digests and subsequent soluble reactive phosphate analysis (Strickland and Parsons 1972). Carbon (C) and N contents were determined using CHN analyses (Perkin-Elmer series 2400). Aliquots (100 µl) of leachate were placed in standard tin capsules (Perkin-Elmer N241-1255), and evaporated (60 °C) until dry and repeated until a total volume of 2 mL were added to the capsules.

Field study

Five parallel 40 m-long transects, running perpendicular to the invasion line (north–south), were established in native prairie in the late fall prior to initiating manipulations the following spring. Transects were at least 5 m apart and a minimum of 8 m from *Sorghum* invaded prairie. Nine 25 × 25 cm plots, spaced approximately 4 m apart each containing a single *Schizachyrium* tussock of similar size (basal diameter ~ 10–25 cm), were established within each transect (N = 45) in a randomized block design (each transect represented one block). Plots within each transect were randomly assigned to create three replicate plots for each experimental treatment: water (DICRO, manipulative control); no water (non-manipulative control); or *Sorghum* leachate (experimental). The manipulative treatments were applied approximately weekly (range 6–12 days) throughout the study period (18 treatments spanning 24 weeks): 3 L of water or leachate were applied to the base of *Schizachyrium* in each plot. Water and leachate were not applied within 24 h of rain.

Upon emergence (end of Mar. 2004), five *Schizachyrium* tillers were tagged on one plant in each plot and height was recorded weekly until harvest on September 7, 2004. Height was taken as the distance from the base to the tip of green tissue. Individual

culms with inflorescences (hereafter, reproductive structures) were counted for each plant upon emergence and height measured weekly until harvest. At harvest, entire plants were removed along with sufficient soil surrounding the plant to ensure roots were collected. Plants were returned to lab facilities where they were excavated intact from soil and rinsed to remove residual material. Plants were separated into two categories and four tissue types: above ground (live leaves and reproductive structures) or below ground (crown bases and roots). All tillers (including those tagged for repeated measures) were grouped per plant and separated as above. Weight was recorded for each tissue after drying for 5 days at 60 °C.

Greenhouse study

In early spring 2004, prior to initiating the field study, soils were removed from the remnant prairie (control/native) and from the invaded area (experimental/*Sorghum* invaded) and returned to lab facilities. Soils from each area were kept separate. All live plant matter, large detritus, and rocks were removed from each soil source and soils were then homogenized. Soil from each source was placed in 1L plastic pots ($n = 16$, $N = 32$) previously soaked for 1 h in 20 % bleach solution, rinsed in DICRO water, and dried.

Dormant *Schizachyrium* plants ($n = 8$) of similar size (basal circumference 10–15 cm) and located within a 2 m radius of one another were removed from native prairie on March 1, 2004, placed in individual pots, and transported to lab facilities. Each of eight plants was divided into six equal clumps to control for potential genetic and inherent root-associated microbial community differences among plants. Soils were removed from each clump and roots were rinsed in DICRO water. For each plant, two clumps were planted into pots containing soil from native prairie, two were planted in soil from invaded prairie, and two were maintained in their original soil to serve as replacements if needed. The plants were clipped (to 5 cm height), and each clump had equal numbers of ramets (four per clump). The plants were acclimated in the greenhouse for 30 days and watered ad libitum prior to treatment. Each plant was treated as a “block”, exposing each clump to one of four treatments ($n = 8$, $N = 32$): native soil/water (NW); native soil/*Sorghum* leachate (NL); invaded soil/water (IW); invaded soil/*Sorghum* leachate (IL).

Five tillers were tagged on each of the 32 plants on April 1, 2004. DICRO water or *Sorghum* leachate (375 mL, respectively, see below) was applied weekly from April 5–July 31, 2004. Tiller height, and reproductive structures upon emergence, were recorded weekly between April 5, 2004 and harvest on August 9, 2004. Plants were harvested using methods outlined in field studies.

Statistical analyses

A randomized block design with repeated measures was utilized to analyze field data measured over the course of the growing season in response to treatment application: tiller height, reproductive structure number and height. Replicate nested in block (transect) was used as an error term for block. For the manipulative field study, biomass at harvest was analyzed separately for each tissue type with a one-way ANOVA (three levels of treatment application: none, water, leachate). A two-by-two factorial design with main effects of soil source (native or invaded) and treatment application (water or leachate) with repeated measures was used to analyze mean tiller height collected from the greenhouse study, while the same two-by-two design without repeated measures was used at harvest for number of sexual reproductive structures, plant height and biomass by tissue type. Post hoc means comparisons were conducted using Tukey’s HSD with $\alpha = 0.05$. Normality and homogeneity of variances were examined and data were log, arcsine, or square root transformed when appropriate. All data were analyzed using SAS for Windows version 8 (SAS Institute, Cary, NC). Biodiversity measures were calculated using the following equations from McCune and Grace (2002): Shannon-Weiner diversity index (H'); $sH' = -\sum p_i \log p_i$ where p_i = proportional abundance of each species (i) calculated from percent cover; and Evenness (e); $e = H'/\log S$; where S = total number of species.

Results

Quantifying invasion progression and defining community structure

Throughout the study period, the *Sorghum* invasion front continued to expand into the native prairie

Table 1 Net progression of *Sorghum* invasion into native tallgrass prairie

Years	Net encroachment (m)
2004–2005	2.31 ± 0.76
2004–2006	2.80 ± 0.89
2004–2007	3.50 ± 0.85
2004–2008	2.74 ± 0.80
2004–2009	2.23 ± 0.69
Mean annual rate	0.45 ± 0.14 (m year ⁻¹)

Values based upon average movement across 37 longitudinal transects, mathematically corrected to reflect meters using the Cosine-Haversine formula. Mean Annual Rate reflects the average rate of invasion advance 2004–2009 calculated on a point-to-point comparison

upwards of at least 2 m based upon comparisons to the invasion line recorded in the initial year (Table 1). Visual representation of the GPS line recorded during the period of experimental manipulations (2004–2006) from the 37 longitudinal points is depicted in Fig. 1. The mean annual invasion rate over the study period (based only on longitudinal movement) was 0.45 ± 0.14 m year⁻¹ (Table 1).

Plant community structure

The presence of three distinct communities, identified as native, transition zone, and invaded was

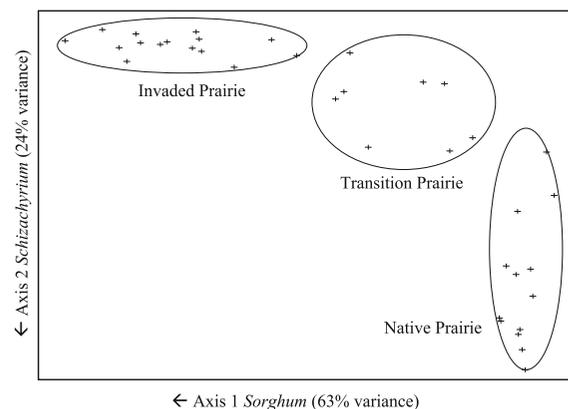


Fig. 1 Principal components analysis on percent cover of 36 1-m² plots differentiated three distinct zones: native (N), invaded (I), and transition (T). Axes 1 and 2 explained 87 % of the variance. Axis 1 had an eigenvalue of 17.43 explaining 63 % of the variance, while axis 2 had an eigenvalue of 6.70 explaining 24 % of the variance. Both showed strong negative loading values associated with a single species: axis 1 with *Sorghum*, (−3.911) and axis 2 with *Schizachyrium*, (−2.445)

confirmed based on percent relative cover (Table 2) as assessed with principal components analysis (PCA, Fig. 1). The native prairie community was dominated by *Schizachyrium*, with more than 20 different species of native forbs and a few grasses each comprising <8 % (Table 2). In contrast, over 48 % the transition zone composition consisted of two species (*Sorghum* and *Schizachyrium*) with *Rubus trivialis* (Southern Dewberry), woody trees, and the invasive grass *Bothriochloa ischaemum* (King Ranch Bluestem) comprising the remaining proportion of the community (Table 2). Composition of invaded prairie displayed a hierarchy, with *Sorghum* overwhelmingly dominant followed by the non-native grass *Bromus japonicus* (Japanese Brom-e), woody trees, and *R. trivialis* (Table 2). Diversity (H') was significantly higher in the transition zone when compared to the other two communities ($F_{11,2} = 23.49$, $p < 0.0001$, Table 2). Evenness was also highest in the transition zone but could only be statistically separated from evenness in the native prairie ($F_{11,2} = 9.83$, $p = 0.0005$, Table 3). Species richness (S) was significantly higher in the native prairie compared to the invaded community ($F_{11,2} = 21.45$, $p < 0.0001$, Table 3).

Invasive plant leachate

Concentrations of nutrients in leachates were very low and varied inconsistently: C concentration ranged approximately fivefold (2.14–10.68 mM), while P and N ranged approximately tenfold (0.025–0.375 mM, 0.055–0.455 mM, respectively).

Field study

Growth

Mean tiller height was similar across all treatments for most of the study period (Fig. 2). However, after 2 months of being slightly larger than controls, leachate-treated plants began to senesce. The height of tillers in control plants (watered and non-manipulative) exceeded that of leachate treated plants after day 142, however this finding was not statistically significant overall (Fig. 2). Since plants grew larger over the course of the study, a significant effect of sampling day was also found ($F_{13,247} = 512.18$, $p < 0.0001$).

Table 2 Percent relative aerial cover of the six most abundant species (or group) in the three prairie communities. Values are mean percentages (\pm 1SE)

Plant species/group	Native (%)	Transition (%)	Invaded (%)
<i>Sorghum halepense</i>	0	27.3 \pm 4.3	76.9 \pm 5.2
<i>Schizachyrium scoparium</i>	58.9 \pm 5.0	21.0 \pm 3.6	0.5 \pm 0.4
<i>Bromus japonicus</i>	1.4 \pm 0.6	5.9 \pm 2.0	20.9 \pm 3.4
<i>Bothriochloa ischaemum</i>	0	11.4 \pm 9.9	2.5 \pm 1.1
<i>Rubus trivialis</i>	1.5 \pm 1.3	16.3 \pm 4.5	9.0 \pm 2.9
Woody shrubs/trees*	0	11.3 \pm 6.6	15.3 \pm 2.8

* This group was comprised of the species *Fraxinus texensis* (Texas ash), *Diospyros virginiana* (Common Persimmon), *Prosopis glandulosa* (Honey Mesquite), and *Melia azedarach* (Chinaberry)

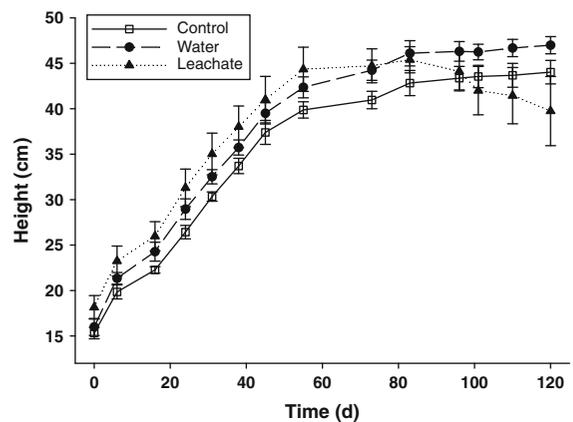
Table 3 Diversity measures for three prairie communities within the study site. Values are means (\pm 1 SE). Different letters denote significantly different means

Field location	Species richness (<i>S</i>)	Shannon–Weiner (<i>H'</i>)	Evenness (<i>e</i>)
Native	9.37 \pm 0.81 ^a	1.17 \pm 0.11 ^b	0.44 \pm 0.05 ^b
Transition	9.00 \pm 0.63 ^a	1.85 \pm 0.06 ^a	0.84 \pm 0.03 ^a
Invaded	4.85 \pm 0.35 ^b	1.09 \pm 0.06 ^b	0.68 \pm 0.04 ^a

Leachate treatments significantly reduced the number of reproductive structures produced by *Schizachyrium* compared to controls (watered and non-manipulative, $F_{2,19} = 16.72$, $p < 0.0001$; Fig. 3). In pairwise comparisons, plants receiving water produced significantly more reproductive structures than plants receiving leachate ($p < 0.0001$, Fig. 3a). Leachate-treated plants failed to increase production of reproductive structures as the growing season progressed, and there were significant effects of transect (block), day, and day \times treatment on abundance of reproductive structures. Additionally, reproductive structures produced by leachate-treated plants tended to be shorter compared to those produced by plants in all other treatments (Fig. 3b), although pairwise comparisons showed this effect was not statistically significant.

Biomass

The manipulations resulted in significant differences in total plant biomass (all $p < 0.05$, Fig. 4). Leachate-treated plants produced less above- and below-ground biomass compared to control plants (water and non-manipulative controls). Both sets of control plants produced equivalent biomass across all tissue types, indicating reduced biomass in leachate-treated plants


Fig. 2 Tiller height of *Schizachyrium* growing in native prairie (Mean \pm 1 SE), $n = 15$ for control and leachate, $n = 14$ for water). Day 0 was March 30, 2004

was a function of materials contained within the leachate, rather than an artifact of the additional water used to deliver the leachate. It is important to note that the mean annual values for the essential macronutrients in the native soils during this same study period were previously reported to be 1.35 ± 0.11 , 13.17 ± 0.66 , and 128.47 ± 4.47 for $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$, and K respectively (see Rout and Chrzanowski, 2009).

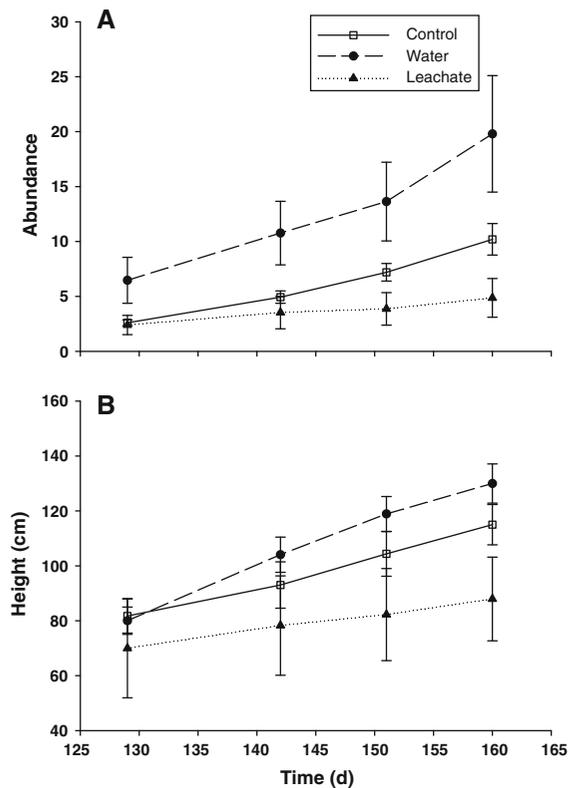


Fig. 3 Abundance (a) and height (b) of reproductive structures (per plant) of *Schizachyrium* growing in native prairie. Day 0 was March 30, 2004. Development of inflorescences began 125 days after monitoring was initiated and measures of abundance and heights were recorded until harvest (day 160). Mean (± 1 SE), $n = 15$ for all treatments except for water where $n = 14$

Greenhouse study

Growth

Mean tiller height of *Schizachyrium* varied as a function of soil source and application type (Fig. 5). There were significant two-way interactions between time (day) and treatment ($F_{13,15} = 4.39$, $p = 0.004$) and between time and soil source ($F_{13,15} = 3.76$, $p = 0.008$). Tillers in all treatment combinations were of approximately equal height at the beginning of the experiment, ranging between 15 and 20 cm (Fig. 5). Plants growing in native soil and receiving leachate (NL) reached a growth plateau after 28 days and began to senesce after 84 days, while plants in soil from the invaded prairie and receiving leachate (IL) grew for a longer time (plateau at 49 days) but senesced earlier (after 70 days). Regardless of soil

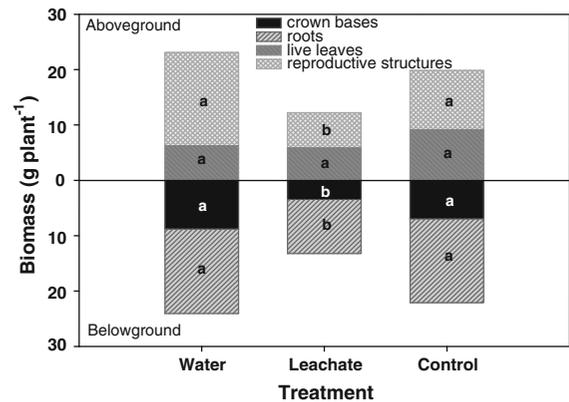


Fig. 4 Biomass of *Schizachyrium* harvested from the manipulative field study after 160 days of treatment. Biomass is separated by tissue type with aboveground tissues appearing above the zero line and belowground tissues below the zero line. Different letters denote significant pairwise differences within tissue type (Mean (± 1 SE), $n = 10$)

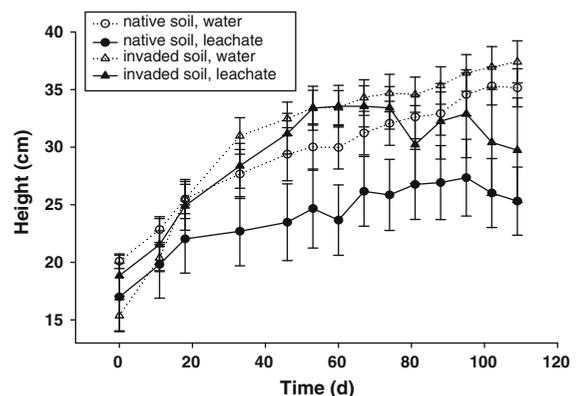


Fig. 5 Tiller height of *Schizachyrium* growing under greenhouse conditions in native prairie soil or in soil previously invaded by *Sorghum*. (Mean (± 1 SE), $n = 8$). Day 0 was April 3, 2004

source, leachate treatment had a significant inhibitory effect on growth by day 84. Over the course of the entire study, and largely due to the different times of onset of senescence, the effect of leachate on plant growth was only marginally significant ($F_{1,27} = 3.65$, $p = 0.07$). After about 30 days plants growing in soils from invaded prairie and receiving water (IW) behaved similarly to plants growing in soils from native prairie and receiving water (NW).

Just as in the field study, application of leachate significantly reduced the number of reproductive structures (Fig. 6a; $F_{1,9} = 14.73$, $p = 0.004$) and their

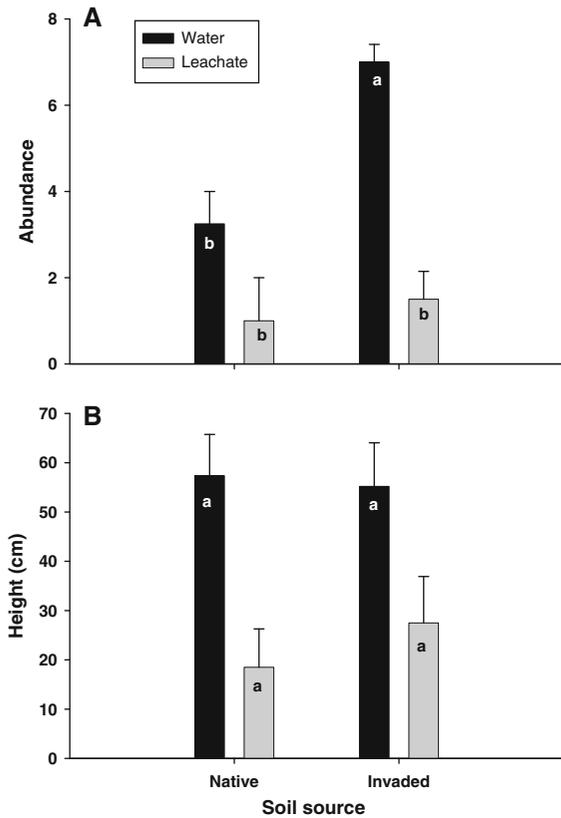


Fig. 6 Abundance (a) and height (b) of reproductive structures of *Schizachyrium* (per plant) growing under greenhouse conditions. Different letters denote significant differences (Mean \pm 1 SE), $n = 4$)

heights (Fig. 6b; $F_{1,9} = 5.57$, $p = 0.04$). Plants in the IW treatment produced significantly more reproductive structures than plants in the other three treatments. Plants receiving leachate growing in both soil sources produced shorter and fewer reproductive structures compared to water-control plants. Pairwise comparisons revealed that plants growing in invaded soils produced significantly fewer reproductive structures than plants growing in native soils (Fig. 5).

Biomass

At the end of the study, total *Schizachyrium* biomass was similar for water-control plants growing in both soil sources, but was significantly reduced in plants receiving leachates (Fig. 7). This was true for all plant tissues [total below ground tissues (roots, crown bases); total above ground tissues (live leaves, reproductive structures)]. Focusing only on leachate-treated

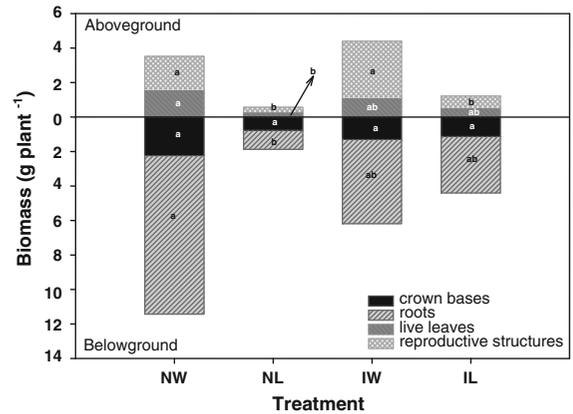


Fig. 7 Biomass of *Schizachyrium* from the manipulative greenhouse study after 128 days of treatment. Biomass is separated by tissue type with aboveground tissues appearing above the zero line and belowground tissues below the zero line. Different letters denote significant pairwise differences within tissue type (Mean \pm 1 SE), $n = 4$). NW, native soil, water; NL, native soil, leachate; IW, invaded soil, water; IL, invaded soil, leachate

Schizachyrium, those growing in invaded-prairie soils were larger than plants growing in native-prairie soils, and this was particularly evident when looking at below ground biomass. While soil source did not have a significant effect on biomass, there was a marginally significant interaction between treatment (leachate) and soil for below ground biomass ($F_{1,9} = 4.67$, $p = 0.06$). Overall, the greenhouse findings showed leachate-treated *Schizachyrium* had significantly less total biomass and reproductive structure biomass compared to plants receiving water (Fig. 7).

Discussion

Invasion impacts on biodiversity

The ability of a community to resist invasion declines dramatically with increased loss of local species, and depends upon the functional role and abundance of native species (Zavaleta and Hulvey 2004). Our work shows that this relationship between abundance and *Sorghum* invasion-resistance exists for *Schizachyrium*. This finding is consistent with previous research linking the native grass to maintaining functional integrity of native prairie communities (Voigt and Weaver 1951). The *Sorghum* invasion-front progression documented in this work is a conservative

measure of the displacement of the native prairie community; regardless, this calculated annual invasion rate ($0.45 \pm 0.14 \text{ m year}^{-1}$) is striking. Conversion of native prairie, a species-rich community dominated by *Schizachyrium* (58.9%), into an invaded community that is approaching a monoculture of *Sorghum* (76.9%), yields an intermediate community in the transition zone where neither grass dominates (see Table 2). Interestingly, the four other species comprising the majority of the cover in the transition zone were absent or minimal in native prairie. Further, two species (*B. ischaemum*, *R. trivialis*) had highest relative cover in the transition zone, but were declining in the invaded prairie, suggesting *Sorghum* can successfully outcompete these species.

It is unclear what factors are causing this shift in the transition community plant composition where neither the native nor the invasive grass dominates, but together comprise <50% of the community (see Table 2). We suggest this might be a short-term effect caused by *Sorghum* modification of the plant-soil feedback environment through expansion of clonal rhizomatous growth prior to establishing dominance (indicated by the invasion-front progression) and subsequent exclusion of the majority of native plants. Rhizomatous, runner clonal plants often have a competitive advantage over bunched growth forms when nutrients are increased or disturbances occur (Gough et al. 2012). It is important to point out that several other invasive plants are establishing inside this transition community, (including *B. ischaemum*, King Ranch Bluestem, and *B. japonicus*, Japanese Brome, see Table 1), that are not establishing in the native prairie, nor are they able to persist in the invaded area. In addition, woody tree invasion into prairie ecosystems (as documented in this work, Table 2) has historically favored declines in grassland communities (Samson and Knopf 1994). Together, this suggests that invasive *Sorghum* might be driving this community alteration process and likely does so through a variety of mechanisms. For example, the modification to the soil nutrient profiles previously documented in this system by members of our group (see Rout and Chrzanowski 2009) indicated increased nutrient availability in this transition compared to the native community, thus potentially opening a niche for invasive and/or weedy species expansion, like those documented here. These findings are consistent with

previous research in tallgrass prairie, which shows that when factors altering the abundance of the dominant grasses are manipulated, competitive release occurs for subordinate grass and forb species, many of which are often invasives (Silletti et al. 2004).

Effects of invasive plant leachates

By harvesting and leaching whole-plants at weekly intervals throughout the growing season, and applying these leachates to comparably sized plots of *Schizachyrium*, we attempted to simulate ecologically-relevant field conditions in a natural community. Materials can be leached or excreted from *Sorghum* at all times during the year, when the plant is actively growing as well as during senescence and decomposition of densely matted culms. One potential limitation of this approach is that leachate created from whole plants may contain sufficient nutrient concentrations to act as fertilizers. Chemical analysis of leachates used here suggested that only very low levels of N and P were contained in the leachates and were not likely to influence experimental outcomes. In addition, water increased growth of *Schizachyrium* above that seen in plants with no addition, suggesting that the effects of the leachates actually counteracted the benefits of adding water to the plants because the suppression of growth was significant. Additionally, findings from the experimental manipulations conducted in the field and greenhouse yielded similar results; compared to controls, plants receiving leachate had significantly reduced reproductive structure biomass, and tended to have less total biomass (as measured by tissue type and whole plant).

Sorghum leachate suppresses vegetative and sexual growth of *Schizachyrium*

In field and greenhouse experiments, negative effects of leachate treatments on tiller heights were observed toward the end of each study in comparison to plants receiving water. Thus, there appeared to be a cumulative effect of materials leached from *Sorghum* on the growth of the target species. Similarly, biomass of vegetative tissues was generally reduced in plants receiving leachate in both the field and the greenhouse. We conclude that material leached from *Sorghum* inhibited vegetative growth of *Schizachyrium*.

Leachate also had a dramatic inhibitory effect on sexual reproductive structures in both field and greenhouse experiments. Regardless of the soil source, abundance and biomass of reproductive structures of *Schizachyrium* were significantly reduced in the presence of *Sorghum* leachate. While there was a trend for height of these reproductive structures to be reduced in the presence of leachate, it was not significant in pairwise comparisons. From these findings we conclude that the significant reduction in reproductive structure biomass was due to the impact of leachates on the quantity of these structures the plant was able to produce, rather than heights of those actually developed. This suggests that materials leached from *Sorghum* inhibit *Schizachyrium* from allocating resources toward sexual reproduction prior to the development of these structures, although this experiment did not directly assess seed production.

The native *Schizachyrium* is a grass that is reliant upon both clonal fragmentation and sexual reproduction via seed for population maintenance and proliferation (Butler and Briske 1988). The evidence from this study suggests that leachates suppress *Schizachyrium* growth both above and belowground, which will likely negatively affect future clonal growth. We observed reduced clonal growth as measured by tiller height and biomass, but we did not directly measure effects on clonal fragmentation in this study. Nevertheless, reduced numbers of reproductive structures likely results in reduced seed production, hence fitness, of adult plants. Further, the reduced height of the inflorescences that were produced may have long-term impacts to the genetic diversity of *Schizachyrium* by potentially limiting wind pollination and seed dispersal.

Negative feedback effect of potential allelochemical legacy in soils

In greenhouse studies, *Schizachyrium* planted in soil originating from invaded prairie initially responded differently from those transplanted into soil from native prairie. However, after 60 days, plants responded similarly to water treatments, regardless of soil source. The known residual time for inhibitory levels of the allelochemical sorgoleone *in soil* is 60 days (Einhelling and Souza 1992; Weston et al. 1989). Based on the results from our greenhouse study, it seems likely that the significant interaction of

soil source and time on the growth of *Schizachyrium* stems from a gradual loss of the allelopathic signal from sorgoleone and its derivatives from invaded soils. This speculation, while seemingly supported by the evidence and the known properties of sorgoleone, will nevertheless require quantitative assessments of sorgoleone degradation in soils before it can be fully addressed, and recent advances in measuring this allelochemical in soils have been made (see Weidenhamer et al. 2009).

Synergistic effects of allelochemicals

Water-soluble leachates created from living plants likely do not contain sorgoleone or the cyanogenic-glycoside dhurrin, although breakdown products of the latter may be present (see Weston et al. 1989). Sorgoleone is a lipid and would not be expected to be extracted from plants by water leaching. Indeed, high performance liquid chromatography of aliquots of leachate failed to reveal the presence of significant levels of sorgoleone (data not shown). Similarly, dhurrin is rapidly degraded following damage to leaf tissue and would not persist in this form in leachates, but would likely convert into *p*-hydroxy benzaldehyde and *p*-hydroxy benzoic acid, both shown to be phytotoxic (Weston et al. 1989). The strong effect of leachate on *Schizachyrium* in both field and greenhouse experiments suggests the presence of allelopathic chemicals readily leached with water from *Sorghum* tissues. Thus, *Sorghum* may alter native plant diversity by allelopathic signals released into soils through root exudation, as our data from the greenhouse study suggests and others have demonstrated, and through water leaching of above ground plants and detritus.

Conclusion

This research is among few studies to quantify the *impact* of an invasive species on the diversity and composition of a native community during invasion progression. Herein, we have contributed to the understanding of how these invader-impacts translate to various detrimental effects on the dominant species (*Schizachyrium*) in a native tallgrass prairie. Our first hypothesis that leachates would negatively affect growth of the native grass was supported. Our findings

indicate that unidentified water-soluble chemicals in *Sorghum* likely contribute to its successful invasion and persistence in a native prairie by displacing *Schizachyrium* through direct effects to growth and reproduction. Our second hypothesis that *Sorghum* invaded soils would inhibit growth of the native grass was not supported, since biomass from water-treated plants growing in invaded soils was similar to those growing in native soils at the end of the study. This finding is likely due to water treatments serving to flush any residual allelochemical legacy out of the invaded soils, as well as the significant increase in several nutrients documented in these invaded soils (Rout and Chrzanowski 2009). Our final hypothesis that the native grass growing in invaded soils and receiving leachate would be the most negatively affected was also not supported. While leachate-treated greenhouse plants were significantly reduced with regard to biomass and reproductive structures, this was most pronounced for those growing in native soils, again likely due in part to the increased nutrient availability in the invaded compared to native soils. Overall, leachates from invasive *Sorghum* inhibited vegetative and sexual growth of the native grass, and this was most pronounced in plants growing in native soils.

Suppression of vegetative and sexual growth of *Schizachyrium* may lead to further losses in the genetic diversity of the native grass at this site. This is important in light of previous findings that indicate as degradation of the tallgrass prairie ecosystem increases, biodiversity and ecosystem services decline, while risk of invasion by exotic species increases (Hoekstra et al. 2005). Our current work documents a significant decline in the native-dominant grass across a *Sorghum* invasion gradient. Previous findings from our group documented shifts in ecosystem services (soil nutrient pools and biogeochemical cycling) across this same invasion gradient (Rout and Chrzanowski 2009). Further research is required to discern the mechanisms by which *Sorghum* alters the plant-soil environment enabling these documented soil biogeochemical changes, which in turn might enable niche expansion for this invasive plant promoting increased abundance as quantified in this study. In light of this, it is likely this successful invader utilizes a variety of mechanisms to achieve community dominance, and further research along these lines conducted across *Sorghum* invasion gradients is needed to

confirm the ubiquity of these results in tallgrass prairie and other ecosystems where *Sorghum* aggressively invades and persists.

Acknowledgments We thank Suzanne Tuttle and Rob Denkhaus of the FWNC&R for their cooperation, assistance and access to field locations. We thank Rima Lucardi, Jeff Knight, and Elizabeth Ramsey for their assistance in the field, with special thanks to Amie Treuer-Kuehn for assistance with statistical analyses and field collections. Thanks to Ray Callaway for editorial comments on an earlier version of this manuscript. Partial funding was provided by the National Science Foundation (Grant 0137832 to LG).

References

- Butler JL, Briske DD (1988) Population structure and tiller demography of the bunchgrass *Schizachyrium scoparium* in response to herbivory. *Oikos* 51:306–312
- Czarnota MA, Paul RN, Dayan FE, Nimbal CI, Weston LA (2001) Mode of action, localization of production, chemical nature, and activity of sorgoleone: a potent PSII inhibitor in *Sorghum* spp. root exudates. *Weed Tech* 15:813–825
- Czarnota MA, Rimando AM, Weston LA (2003) Evaluation of root exudates of seven sorghum accessions. *J Chem Ecol* 29:2073–2083
- D’Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Ann Rev Ecol Sys* 23:63–87
- Diggs GM Jr, Lipscomb BL, O’Kennon RJ (1999) Shinnery & Mahler’s illustrated flora of North Central Texas. Botanical Research Institute of Texas, Fort Worth
- Einhelling FA, Souza IF (1992) Phytotoxicity of sorgoleone found in grain sorghum root exudates. *J Chem Ecol* 18:1–11
- Gonzalez VM, Kazimir J, Nimbal CI, Weston LA, Cheniae GM (1997) Inhibition of photosystem II electron transfer reaction by the natural product sorgoleone. *J Agri Food Chem* 45:1415–1421
- Gordon DR (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. *Ecol Appl* 8:975–989
- Gough L, Gross KL, Cleland EE, Clark CM, Collins SL, Fari-gone JE, Pennings SC (2012) Incorporating clonal growth form clarifies the role of plant height in response to nitrogen addition. *Oecologia*. doi:10.1007/s00442-012-2264-5
- Halkier BA, Moller BL (1989) Biosynthesis of the cyanogenic glucoside dhurrin in seedlings of *Sorghum bicolor* (L.) Moench and partial purification of the enzyme system involved. *Plant Phys* 90:1552–1559
- Hejl AM, Koster KL (2004) The allelochemical sorgoleone inhibits root H⁺ - ATPase and water uptake. *J Chem Ecol* 30:2181–2191
- Hierro JL, Callaway RM (2003) Allelopathy and exotic plant invasion. *Plant Soil* 256:29–39
- Hoekstra JM, Boucher TM, Ricketts TH, Roberts C (2005) Confronting a biome in crisis: global disparities of habitat loss and protection. *Ecol Lett* 8:23–29

- Inderjit, Callaway RM (2003) Experimental designs for the study of allelopathy. *Plant Soil* 256:1–11
- Inderjit, Kaur M, Foy CL (2001) On the significance of field studies in allelopathy. *Weed Tech* 15:792–797
- Jang CS, Kamps TL, Skinner DN, Schulze SR, Vencill W, Paterson AH (2006) Sorghum genes with rhizome-enriched expression: functional classification, genomic organization, putative cis acting regulatory elements, and relationship to QTLs. *Plant Physiol* 142:1148–1159
- Kagan IA, Rimando AM, Dyan FE (2003) Elucidation of the biosynthetic pathway of the allelochemical sorgoleone using retro biosynthetic NMR analysis. *J Biol Chem* 278:28607–28611
- Knopf FL, Scott ML (1990) Altered flows and created landscapes in the Platte River headwaters, 1840–1990. In: Sweeney JM (ed) *Management of dynamic ecosystems*. North Central Section of the Wildlife Society, Indiana, pp 47–70
- Levine JM, Vilv M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proc R Soc Lond* 270:775–781
- Liao C, Peng R, Luo Y, Zhou X, Wu X, Fang C, Chen J, Li B (2008) Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta-analysis. *New Phytol* 177:706–714
- McCune B, Grace JB (2002) Species diversity. In: McCune B, Grace JB (eds) *Analysis of ecological communities*. MJM Press, Oregon, pp 25–26
- McWhorter CG (1961) Morphology and development of johnsongrass plants from seeds and rhizomes. *Weeds* 9:558–562
- McWhorter CG (1981) Johnson grass as a weed. *USDA Farm Bull* 1537:3–19
- Meazza G, Scheffler BE, Tellez MR, Rimando AM, Romagni JG, Duke SO, Nanayakkara D, Khan IA, Abourashed EA, Dyan FE (2002) The inhibitory activity of natural products on plant *p*-hydroxyphenylpyruvate dioxygenase. *Phytochemistry* 60:281–288
- Nielsen JS, Moller BL (1999) Biosynthesis of cyanogenic glucosides in *Triglochin maritima* and the involvement of cytochrome P450 enzymes. *Arch Biochem Biophys* 368:121–130
- Nimbal CI, Pedersen JF, Yerkes CN, Weston LA, Weller SC (1996) Phytotoxicity and distribution of sorgoleone in grain sorghum germplasm. *J Agri Food Chem* 44:1343–1347
- Paterson AH, Schertz KF, Lin YR, Liu SC, Chang YL (1995) The weediness of wild plants: molecular analysis of genes influencing dispersal and persistence in johnsongrass, *Sorghum halepense* (L.) Pers. *Proc Nat Acad Sci* 92:6127–6131
- Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. *Bioscience* 50:53–65
- Prach K, Pyšek P (1994) Clonal plants—what is their role in succession? *Folia Geobot Phytotax* 29:307–320
- Pyšek P (1997) Clonality and plant invasions: can a trait make a difference? In: de Kroon H, van Groenendael J (eds) *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, pp 405–427
- Rimando AM, Dyan FE, Czarnota MA, Weston LA, Duke SO (1998) A new photosystem II electron transfer inhibitor from *Sorghum bicolor*. *J Nat Prod* 61:927–930
- Robusto CC (1957) The cosine-haversine formula. *Am Math Mon* 64:38–40
- Rout ME, Callaway RM (2009) An invasive plant paradox. *Science* 324:724–725
- Rout ME, Callaway RM (2012) Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that 'everything is *not* everywhere'. *Ann Bot* 110:213–222
- Rout ME, Chrzanowski TH (2009) The invasive *Sorghum halepense* harbors endophytic N₂-fixing bacteria and alters soil biogeochemistry. *Plant Soil* 315:163–172
- Samson F, Knopf F (1994) Prairie conservation in North America. *Bioscience* 44:418–421
- Silletti AM, Knapp AK, Blair JM (2004) Competition and coexistence in grassland codominants: responses to neighbour removal and resource availability. *Can J Bot* 82:450–460
- Strauss SY, Webb CO, Salamin N (2006) Exotic taxa less related to native species are more invasive. *Proc Nat Acad Sci* 103:5841–5845
- Strickland JHD, Parsons TR (1972) *A practical handbook of seawater analysis*, 2nd ed. *Bull Fish Res Board Can* 167:1–30
- Vitousek PM, D'Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *New Zeal J Ecol* 21:1–16
- Voigt JW, Weaver JE (1951) Range condition classes of native Midwestern pasture: an ecological analysis. *Ecol Monogr* 21:39–60
- Weidenhamer JD, Boes PD, Wilcox DS (2009) Solid-phase Root Zone Extraction (SPRE): a new methodology for measurement of allelochemical dynamics in soil. *Plant Soil* 322:177–186
- Weston LA, Harman R, Mueller S (1989) Allelopathic potential of sorghum-sundangrass hybrid (sudex). *J Chem Ecol* 15:1855–1865
- Wilcove DS, Rothstein D, Dubow J, Phillips A, Loso E (1998) Quantifying threats to imperiled species in the United States. *Bioscience* 48:607–615
- Wilson SD, Tilman D (1993) Plant competition and resource availability in response to disturbance and fertilization. *Ecology* 74:599–611
- Zavaleta ES, Hulvey KB (2004) Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science* 306:1175–1177