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REGULAR ARTICLE

The invasive *Sorghum halepense* harbors endophytic N_2-fixing bacteria and alters soil biogeochemistry

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Abstract Exotic plants invading new habitats frequently initiate broad changes in ecosystem functioning. *Sorghum halepense* is an invasive grass capable of growing in nitrogen (N)-poor tallgrass prairie soils that creates near monocultures in once phylogenetically diverse-communities. The biogeochemistry of soils invaded by *S. halepense* was compared to that of un-invaded native prairie soils. Invaded soils contained two to four times greater concentrations of alkaline metals, micronutrients, and essential plant nutrients than native prairie soils. The notable exception was Ca^{+2}, which was always significantly lower in invaded soils. The N-content of *S. halepense* above-ground biomass was 6.4 mg g^{-1} (320 mg N $plant^{-1}$) and suggested a supplemental N source supporting plant growth. Altered soil biogeochemistry in invaded areas coupled with high above-ground biomass in N-poor soils suggested N_2-fixing activity associated with *S. halepense*. Nitrogenase activity of plant tissues indicated that N_2-fixation was occurring in, and largely restricted to, *S. halepense* rhizomes and roots. A culture approach was used to isolate these N_2-fixing bacteria from plant tissues, and 16S rRNA gene sequencing was used to identify these bacterial isolates. Nitrogenase activity of bacterial isolates indicated several were capable of N_2-fixation. In addition to N_2-fixation, other roles involved in promoting plant growth, namely mobilizing phosphorus and iron chelation, are known for closest matching relatives of the bacterial isolates identified in this work. Our results indicate that these plant growth-promoting bacteria may enhance the ability of *S. halepense* to invade and persist by altering fundamental ecosystem properties via significant changes in soil biogeochemistry.

Keywords N_2-fixing bacteria · Endophytes · Invasive plants · Soil biogeochemistry · *Sorghum halepense*

Abbreviations
PBS phosphate buffered saline
ARA acetylene reduction assay

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Introduction

The modification of resource availability (here broadly considered as nutrient cycling) by invasive plants is well documented (Vitousek and Walker 1989; Evans et al. 2001; Kourtev et al. 2002; Ehrenfeld 2003; Reinhart and Callaway 2006) and considerable attention has been focused on nitrogen (N) cycling, one of

the most basic of ecosystem processes (Vitousek et al. 1987; Stock et al. 1995; Yelenik et al. 2004). In many cases, an invasive or newly introduced species may modify the habitat by simply adding a new functional trait to the ecosystem; for example, introduction of the N-fixing tree *Myrica faya* into N-limited volcanic soils where there were no or few native N-fixing plants introduced a completely novel ecosystem process, which subsequently modified the habitat (Vitousek 1986; Vitousek and Walker 1989). Alternatively, an invasive or newly introduced species may not introduce a new functional trait, but may instead alter existing nutrient cycles. For example, in ecosystems supporting a variety of N_2-fixing plants, invaders altering the N-cycle may bring about new interactions with soil microbial communities (Reinhart and Callaway 2006) or modify existing feedback loops (Klironomos 2002) that affect species diversity and overall ecosystem functioning. In either case, the changes in nutrient cycling brought about by invasive plants shed new light on the mechanisms leading to successful invasions.

Sorghum halepense is a globally distributed allelopathic invasive-grass that resists displacement once established (Holm et al. 1977, Bais et al. 2006). This successful invader has many properties which suggest that it has a high N-demand and may impact the soil biogeochemistry of areas it invades. Most obvious among these properties is the presence of the N-rich constitutive-defense chemical, dhurrin, contained within leaves. In addition to containing dhurrin, *S. halepense* also has considerable above-ground biomass; it often grows as a densely packed monoculture, achieving an abundance of >90 ramets m^{-2} (Rout 2005) and exceeding 2 m in height (McWhorter 1981). In plants harvested from Fort Worth Prairie (tallgrass prairie, south central USA), the N-content of *S. halepense* above-ground biomass was 6.4 mg g^{-1}, or 320 mg N $plant^{-1}$ (Rout, unpublished data). Across the southern portions of the USA, *S. halepense* establishes and expands rapidly; surprisingly, even in soils that are exceptionally N-poor (as in Fort Worth Prairie soils where total N is ~0.008 g kg^{-1}, Rout 2005). It seems paradoxical that this highly productive grass can persist and expand in N-poor soils. The N-content of the plant, when combined with large above-ground biomass and high abundance, suggests that *S. halepense* alters the N-availability, and consequently N-cycling, in areas in which it invades.

In this work we investigated the role of *S. halepense* invasion on soil biogeochemistry. We measured soil nutrient pools in tallgrass prairie invaded by *S. halepense* and compared them to soil nutrient pools in adjacent native-prairie. Dramatic differences in the pools of several elements, particularly N as nitrate (NO_3^-) between native and invaded prairie, prompted us to explore the hypothesis that this invasive grass harbors N_2-fixing bacteria. We present the first evidence of the presence of N_2-fixing and other plant growth-promoting bacteria living endophytically within *S. halepense* rhizomes and those closely associated with roots. Based on the presence of these bacteria and the changes in nutrient pools of invaded soils, we present a speculative model describing a positive nutrient-cycling feedback loop based on N-cycling that might explain the modification of resource availability and bring about the observed changes in the biogeochemical environment.

Materials and methods

Study site

The ~1,400 ha Fort Worth Nature Center and Refuge (32°84′ N, 97°47′ W, FWNCR) is located in Fort Worth, TX, USA. The refuge stretches across an ecotone between Fort Worth (Grand) Prairie and Western Cross Timbers. Fort Worth Prairie is tallgrass prairie characterized by the dominant vegetation, *Schizachyrium scoparium* (little bluestem, Diggs et al. 1999), and has a shallow-gravel alkaline clay-based soil underlain by limestone (hereafter, native prairie). *S. scoparium* is a C_4 perennial caespitose grass that relies on clonal fragmentation and sexual reproduction for population maintenance and dispersal (Butler and Briske 1988), obtains soil phosphorus by being an obligate mycotroph (Anderson et al. 1994), and has never been reported to fix N_2. A section of the prairie (~12 ha) is undergoing extensive invasion by *S. halepense* (hereafter, invaded prairie). Historically (~25 years ago), this site was a contiguous native prairie remnant, devoid of the invasive *S. halepense* (Tuttle, FWNCR, personal communication). Now, there is a clear shift in the vegetation of the ecosystem: *S. halepense* is advancing as a distinct invasion wave and displacing the dominant native flora (Rout 2005). The invasion of *S. halepense* into native prairie creates a transition zone between invaded and native prairie that is characterized by *S. halepense* emergence from

rhizomes in areas where the plant was not previously found. Along some sections of the invasion front, *S. halepense* has advanced into native prairie at a rate of approximately 3 m $year^{-1}$ (Rout 2005). We functionally defined the transition zone as that area between native and invaded prairie where *S. scoparium* and *S. halepense* were equally abundant based on percent cover (data not shown).

Soil nutrient analyses

Soil analyses were conducted on soils collected quarterly spanning a 1-year period; November 2006, through November (inclusive) 2007. Soil cores (three to five discrete, ~3 cm diameter, 13 cm deep) were collected from replicate 1 m^2 plots (n=4) established within native-prairie, invaded-prairie, and the transition zone (N=12). Cores from each plot were pooled into a composite sample, air dried, homogenized, and plant debris removed. Soil analyses of pH, exchangeable alkaline metals (Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}), micronutrients (Zn^{2+}, Fe^{3+}, Cu^{+}, and Mn^{2+}), and essential plant nutrients (N as NO_3^{2-} and phosphorus as available PO_4^{3-}) were conducted commercially (Texas A&M University Soils Lab). Brief descriptions of the methods used by this facility are available at http://soiltesting.tamu.edu/.

Nitrogenase activity associated with *S. halepense* tissues

Rhizomes were harvested from random areas of invaded prairie in February 2006, planted in an initially sterile sand–vermiculite mix, and grown under greenhouse conditions until plants attained a height of ~30 cm (~90 days). Leaves, roots, and rhizomes (0.25–0.75 g) were rinsed in sterile water (Milli-Q) to remove loose soil and surface debris. Tissues were surface sterilized (30 s sequentially in 1% Chloramine-T detergent solution, 95% ethanol, and 1.6% hypochlorite; tissues were rinsed 3x with sterile water between each step). The outer, coarse surface layer of rhizomes was removed after surface sterilization, leaving only tissue that did not come in contact with the soil. Plant tissue was aseptically transferred to glass tubes (16×150 mm) containing 200 μL sterile water and fitted with serum stoppers. Air (10% of headspace) was removed from each tube, replaced with an equal amount of acetylene gas, and incubated (26°C, 24 h). Acetylene conversion to ethylene (ARA) was determined using gas chromatography (SRI Instruments). Tubes without plant tissue, but injected with acetylene, served as controls. This procedure was repeated on rhizomes harvested from three randomly selected areas of invaded prairie in April 2008. Segments of individual rhizomes (0.6–2.0 g) were rinsed, surface sterilized, and outer tissue layers removed (as detailed above), and assayed for nitrogenase activity using ARA (Shimadzu GC-2014). An additional set of controls (in triplicate) were used in this assay to control for endogenous ethylene production, which consisted of tubes containing rhizomes without acetylene injection. We also examined the potential for nitrogenase activity in the native prairie grass, *S. scoparium* using root tissues of comparable weights to those used for *S. halepense* rhizomes.

Isolation and identification of bacteria associated with *S. halepense* tissues

Three ~30-cm diameter, 15-cm deep plots of *S. halepense* were harvested on two occasions during the summer (2006) from separate, randomly selected areas of invaded prairie and transported to nearby laboratory facilities. Tissues (~0.25 g of leaves, roots, and rhizomes) were surface sterilized, and ground (sterile mortar and pestle) in 1 mL sterile phosphate buffered saline (PBS). Outer surfaces of rhizomatous tissues were removed (detailed above) after surface sterilization. Extracts from each tissue type were diluted (10^{0}–10^{-7} in PBS in 10^{-1} steps) and 0.1 mL of each dilution was inoculated into N-free semisolid medium (JNFb, Baldani et al. 1996) and incubated (30°C, 4 days). Turbidity or distinct below-surface veils or pellicles were taken to represent growth. Subsamples of cultures showing positive growth were plated (0.1 mL) onto JNFb yeast-extract agar (Baldani et al. 1996), colonies isolated (2 days growth), and repurified. Isolates were grouped by colony characteristics to yield 19 distinct morphotypes. Of these, the five that were most frequently isolated from plant tissues (i.e. the most frequently recovered) were identified by 16S rRNA gene sequencing.

Bacterial genomic DNA for comparative sequence analysis was extracted from pure cultures using the Ultra Clean Microbial DNA Isolation Kit (MoBio). Approximately 1,400 nt of the 16S rRNA gene was

amplified using the general bacterial primers, 27f (AGAGTTTGATYMTGGCTCAG) and 1492r (TACGGYTACCTTGTTACGACT). PCR products were purified (Ultra Clean PCR Clean-Up Kit, MoBio) to remove dNTPs and non-target DNA, and sequenced using the AB3130xl Genetic Analyzer (Applied Biosystems) at the University of Montana, Murdock DNA Sequencing Facility. Phylogenetic analysis was performed using Laser Gene DNAStar software version 5.01 and sequences were aligned using the Ribosomal Database Project II (RDP, Cole et al. 2007) SeqMatch program incorporating closely related sequences from GenBank (http://www.ncbi.nlm.nih.gov/entrez).

Nitrogenase activity associated with bacterial isolates

Pure cultures of isolates were each inoculated into JNFb medium and incubated (30°C, 4 days) prior to ARA measurements (Shimadzu GC-2014) using methods described above, with the exception of incubation time (1 h). Uninoculated JNFb medium injected with acetylene served as negative controls. *Hebaspirillum seropedicae* (ATCC 35892) was used as a positive control.

Statistical analyses

Soil biogeochemistries were analyzed separately with one-way Analyses of Variance (ANOVA; three levels of treatment application: native prairie, transition zone, invaded prairie). Normality and homogeneity of variances were examined and data were transformed when appropriate. Kruskal–Wallis ANOVA on ranks was applied when data failed normality or equal variance tests. Post hoc means comparisons were conducted using Tukey's HSD (ANOVA) or by Dunn's method (Kruskal–Wallis). All statistical analyses were conducted using SigmaStat 3.11 (Systat Software, Inc., San Jose, CA, USA).

Results

Soil nutrient analyses

Soils collected from within each prairie type were similar throughout the entire sampling period; in most cases the variability associated with alkaline metals, micronutrients, and essential plant-nutrients was less than 10% of the mean (Fig. 1). Invaded and transition-zone soils had pH values slightly, but significantly, lower than those of native soils. The nutrients Mg^{2+}, K^{+}, Zn^{2+}, Fe^{3+}, Mn^{2+}, NO_3^{2-} and, PO_4^{3-} were significantly greater in invaded soils compared to native soils. In most cases, the concentration of a nutrient element was two to fourfold greater in invaded soils than in native soils. The concentration of several nutrients increased progressively from native prairie through the transition zone and into fully invaded prairie (Fig. 1). Calcium was the only nutrient element significantly lower in invaded prairie than in native prairie. This pattern remained consistent throughout the study period and low standard errors of the means indicate that the observed patterns were not the result of a single pulse-event.

Nitrogenase activity associated with *S. halepense* tissues

The ARA indicated strong nitrogenase activity was associated with rhizomes collected from plants grown in the greenhouse (86% positive, max=27 μmol C_2H_4 g^{-1} day^{-1}, n=7, Table 1). Greenhouse grown plants also showed nitrogenase activity was associated with roots (57% positive, max=3 μmol C_2H_4 g^{-1} day^{-1}, n=7) and leaves (23% positive, max=0.9 μmol C_2H_4 g^{-1} day^{-1}, n=4), but the signal was variable and low compared to activity associated with rhizomes. Similarly, the ARA indicated nitrogenase activity associated with field collected rhizomes (85% positive, n=13). Nitrogenase activity was variable and rates of acetylene reduction were similar to those obtained from rhizomes collected from greenhouse grown plants. Nitrogenase activity ranged between 0 and 3.9 μmol C_2H_4 g^{-1} day^{-1} and the activity of nitrogenase positive rhizomes averaged 0.97 μmol C_2H_4 g^{-1} day^{-1} (n=11). No nitrogenase activity was detected for the native prairie grass *S. scoparium* (n=6, data not shown).

Isolation and identification of bacteria associated with *S. halepense* tissues

Bacteria capable of growth in N-free medium were isolated from leaves, roots, and rhizomes. We cultured 54 isolates which were grouped by colony characteristics into 19 distinct morphotypes. Of all 54 original

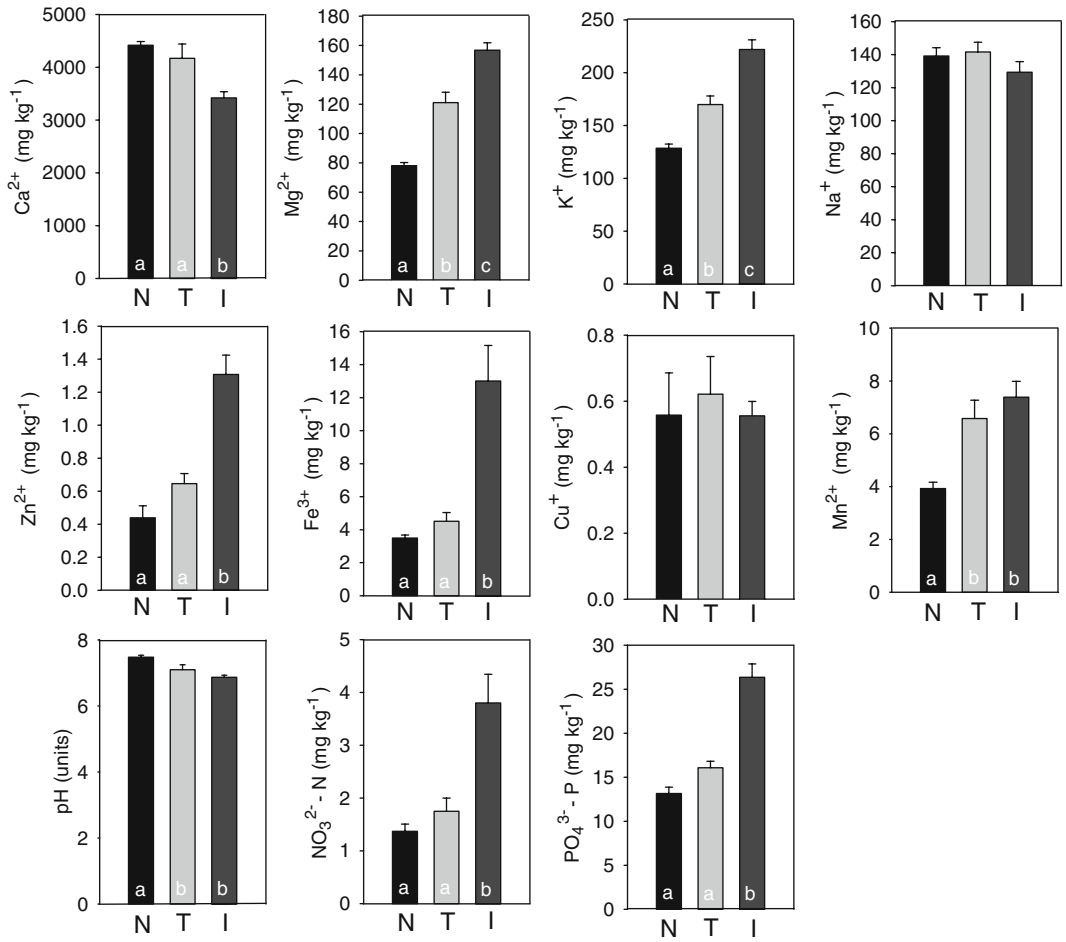

Fig. 1 Mean concentrations of various mineral nutrients extracted from soils collected in native, transition-zone, and invaded prairie. Analyses of Variance indicated that, with the exception of Na+ and Cu+, concentrations of various elements were significantly different among the soil types (F values between 16.2 and 64.6, p always <0.001: *H* values between 11.9 and 38.8, *p* always <0.003). Pairwise comparisons of the means are indicated by *letters appearing at the base of columns* in each figure. Statistically different means are indicated by *different letters*

isolates, the majority were isolated from rhizomes (56%) and roots (41%). We selected five of the 19 morphotypes to be identified using 16S rRNA gene sequencing. These five morphotypes were chosen because they contained over 50% of the 54 original isolates. Their identities, the plant tissues they were isolated from, and the percent similarity match are given in Table 2. A similarity score reports the percent sequence identity over all pairwise comparable positions when aligned with RDP sequences. The percent similarity matches listed are all >97% to type strain isolates of near-full-length sequences (≥1,200 bp).

Table 1 Nitrogenase activity associated with various *S. halepense* tissues

Year	Plant location	Growing season: tissue	Nitrogenase activity (μmol C_2H_4 mL^{-1} day^{-1})
2006	Greenhouse	Early: rhizomes	29.5±0.68, *n*=7
		Late: rhizomes	0.60±0.25, *n*=7
		Late: roots	1.01±0.83, *n*=7
		Late: leaves	0.06±0.02, *n*=4
2008	Field	Early: rhizomes	0.97±0.41, *n*=11

Table 2 Bacteria associated with tissue types of *S. halepense*

Tissue	Isolate	Similarity score	GenBank type strain	Notes on potential ecological role
Rhizomes	*Xanthomonas melonis,* strain LMG8670	99.6	Y10756	Plant pathogen[a]
Rhizomes	*Agrobacterium tumefaciens*	99.3	M11223	Plant pathogen[b], organism fixes N[b]
Rhizomes – roots	*Sphingobium amiense,* strain YT	98.2	AB047364	N_2-fixation in genus[c], nonylphenol degrader[d]
Rhizomes	*Pseudomonas jessenii,* strain CIP105274	99.6	AF068259	N_2-fixation in genus[e], respires NO_3[e], produces Fe-siderophores[e], solubilizes PO_4[e]
Rhizomes	*Caulobacter vibroides* strain (ATCC) 15252T	99.1	AJ227756	Horizontal gene transfer reported with N_2-fixing organisms[f]

All submitted sequences were >1,300 bp.

[a] Vauterin et al. (1992)

[b] Kanvinde and Sastry (1990)

[c] Xie and Yokota (2006)

[d] Ushiba et al. (2003)

[e] Garrity et al. (2005)

[f] Wong and Golding (2003)

Given the sequences obtained in this work were near-full-length 16S rRNA sequences (1,323–1,445 bp), but were not perfect matches to the known type strains, it is possible that the bacterial isolates we obtained may be unique strains (Fox et al. 1992; Stackebrandt and Goebel 1994). We intend to fully characterize these isolates in future research.

Nitrogenase activity associated with bacterial isolates

The ARA indicated nitrogenase activity associated with three of the five bacterial isolates recovered from plant tissues (Table 3). Positive rates of nitrogenase activity ranged between 0.144 and 0.480 nmol C_2H_4 mL^{-1} h^{-1}; these rates range between 25% of, to greater than 100% of those recorded for the positive control (*H. seropedicae*). All bacterial isolates had turbidity (distinct below-surface veils or pellicles) at the time of the ARA. Nitrogenase activity for negative controls (2) were averaged and subtracted from all ARA measurements reported for the bacterial isolates in Table 3. Since the closest match relatives of several isolates showing positive results for nitrogenase activity have not been previously reported as N_2-fixing organisms, it is likely the isolates obtained in this work may be unique strains.

Discussion

There is a clear shift in the plant community structure of prairie ecosystems invaded by *S. halepense*. Growing as virtual monocultures, this grass rapidly displaces native plant communities in Fort Worth

Table 3 ARA nitrogenase activity on bacterial isolates recovered from *S. halepense* tissues

Isolate	Nitrogenase activity (nmol C_2H_4 mL^{-1} h^{-1})
Herbaspirillum seropedicae[a] strain (ATCC)35892	0.411
Agrobacterium tumefaciens	0.007
Xanthomonas melonis, strain LMG 8670	0.003
Sphingobium amiense strain YT	0.144
Pseudomonas jessenii, strain CIP105274	0.480
Caulobacter vibroides, strain (ATCC) 15252T	0.281

All values reported reflect calculations after accounting for negative controls.

[a] This organism served as the positive control.

Prairie (Rout 2005). Our work shows that prairie soils associated with *S. halepense* also undergo dramatic changes in resource availability resulting in increased concentrations of alkaline metals, micronutrients, and essential plant-nutrients (Fig. 1). Interestingly, ANOVA revealed that the biogeochemical signatures of invaded prairie soils often differed significantly from that of native prairie soils despite their close proximity (<50 m) and shared geologic origins. The shift in biogeochemical signatures of these soils may be a consequence of organic acids released by *S. halepense* through root-rhizome exudates (see Bais et al. 2006); however, the N required to support the biomass of this plant suggests involvement of microbial processes associated with the N-cycle, specifically nitrification. We initially suspected that *S. halepense* invasion modified the habitat to promote the growth of free-living N_2-fixing bacteria which would subsequently supply the plant with the necessary N to support growth. However, a preliminary experiment using enrichment cultures suggested that free living N_2-fixing bacteria were lower (3×) in invaded soils compared to native soils (data not shown). An alternative source of N to support *S. halepense* growth could be from bacteria associated with the formation of nodules (primarily seen in legumes). There are many non-legume species that can form nodules when in association with the actinomycete, *Frankia* (Lambers et al. 1998) as well as one non-legume taxon, *Parasponia*, that forms nodules when it associates with *Rhizobium* (LaFay et al. 2006). Since *S. halepense* is a non-leguminous plant that does not show the presence of nodules, we explored the possibility that it is associated with endophytic N_2-fixing bacteria, which have often been found within grasses (Reinhold-Hurek and Hurek 1998).

Other grasses are known to harbor N_2-fixing bacteria belonging to the genus *Herbaspirillum* (Kirchhof et al. 2001). *Sorghum bicolor*, one of the hybridization parents of *S. halepense*, harbors bacteria of this genus as endophytes (Baldani et al. 1996; James et al. 1997; Kirchhof et al. 2001). Thus it seemed likely that members of the genus *Herbaspirillum* would be associated with *S. halepense*. The culture techniques we utilized were specifically designed to recover members of the *Herbaspirillum* genus; however, *Herbaspirillum* was not recovered from *S. halepense*. We further probed for members of this genus via molecular techniques. DNA was extracted from rhizomes that were surface sterilized, as well as from those that were not surface sterilized, and probed for *Herbaspirillum* using the genus specific primer HERB68 (AGCAAGCTCCTATGCTGC) coupled with the general reverse primer 907r (Feris et al. 2003). Again, *Herbaspirillum* was not detected. It is possible that members of the *Herbaspirillum* genus were present, but at concentrations below detection. Additionally, plant phenolic compounds may have inhibited amplification of *Herbaspirillum* in PCR amplifications.

The presence of endophytic and closely associated root N_2-fixing bacteria was confirmed by nitrogenase activity associated with *S. halepense* rhizomes, roots, and pure culture bacterial isolates from these tissues. In the case of rhizomes, nitrogenase activity was confirmed in both greenhouse grown plants and plants collected directly from field sites. While the absolute rates of nitrogenase activity may be biased (see caution below), we were able confirm (both by culture methods and ARA) that N_2-fixing bacteria were closely associated with plant roots and were living endophytically within rhizomes. Some caution must be applied when interpreting rates of nitrogenase activity associated with plant tissues since relatively long incubation times were used for these analyses (James 2000 and citations therein). We utilized shorter incubation times when assessing nitrogenase activity of bacterial isolates in pure culture (1 h). Surprisingly, the known N_2-fixing isolate *Agrobacterium tumefaciens*, did not demonstrate nitrogenase activity when growing in JNFb medium. Nitrogenase activity was assessed on four day old cultures; thus, it is possible that the cultures capable of growth on the N-free medium but not testing positive for nitrogenase activity (*A. tumefaciens* and *Xanthomonas melonis*) were no longer actively fixing N_2 at the time of the ARA. Collectively, these findings suggested the presence of N_2-fixing organisms within the rhizomes of *S. halepense*; no such N_2-fixation has been reported for this invasive grass until now.

We did not seek to exhaustively examine the microflora associated with the plant. It appears that N_2-fixing bacteria are associated with *S. halepense* roots and rhizomes and these bacteria seem to be actively fixing N_2 (indicated by the ARA on pure cultures) and altering the N-cycle in invaded areas. It is likely that additional N_2-fixing bacteria were associated with the plant tissues and that these

bacteria were not isolated by culturing, or were overlooked by our screening to simply detect and select the most common N_2-fixing bacteria. In addition to N_2-fixation, several interesting ecological roles are known for the closest matching organisms of the bacterial isolates recovered. These ecological roles include iron siderophore production, the ability to solubilize phosphate, and plant pathogenicity (listed in Table 2).

Through its association with bacteria, *S. halepense* appears to alter plant species diversity and resource availability, which subsequently modifies the habitat within the remnant tallgrass prairie. Unlike the situation where a newly introduced species adds a new functional trait to the ecosystem, the attribute of N_2-fixation in *S. halepense* does not introduce a new functional trait into this prairie ecosystem. Several native plant species harboring nodulating N_2-fixing bacteria co-exist in Fort Worth Prairie, including the tree *Prosopis glandulosa*, the forbs *Chamaecrista fasciculata*, *Indigofera miniata*, and several members of the *Lupinus* genus. Tallgrass prairie ecosystems are typically dominated by a few grass species and have a higher proportion of diversity resulting from forbs that co-exist in low abundance (Gotelli and Simberloff 1987; Collins and Glenn 1990). Yet, *S. halepense* displaces this diverse native plant community along with the dominant grass species (percent cover 80%), *S. scoparium* (Rout 2005). Using the ARA, we confirmed the native *S. scoparium* demonstrated no N_2-fixation compared to *S. halepense*. This result suggests associations with N_2-fixing bacteria substantially contribute to the biomass (N content) of *S. halepense*, which may play a role in the rapid expansion into the native prairie. It will be necessary to confirm this notion with ^{15}N methods to assess the proportion of plant N derived from biological N_2-fixation. Our work also shows alterations to resource availability coincide with heavily invaded areas. Clearly, the plant or the plant–microbe interaction brings about a change in the habitat, both to the above-ground community and in below-ground processes.

The competitive ability of *S. halepense* (through the plant N-requirements) may be enhanced by microbial activities of closely associated bacteria; the secondary microbial processes may condition soils and favor the persistence of this successful invader. A conceptual model of these processes is shown in Fig. 2. This conceptual model focuses on

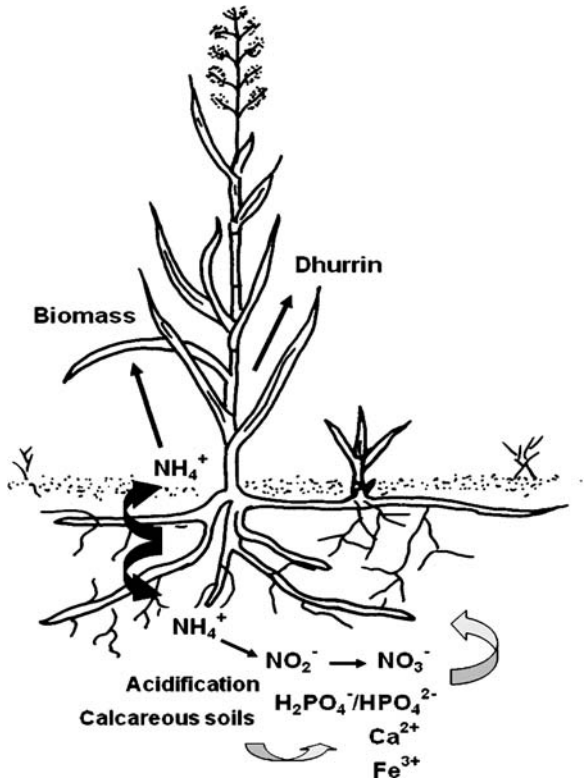

Fig. 2 A conceptual model of microbial processes occurring in the rhizosphere of *S. halepense*. Rhizosphere N_2 is fixed by endophytic N_2-fixing bacteria closely associated with rhizomes. NH_4^+ is shuttled into plant biomass and dhurrin synthesis. Some NH_4^+ is lost to the rhizosphere and converted rapidly into NO_3^-, the preferred form of external N for *S. halepense*. A portion of the NO_3^- remains in the soil, accounting for higher soil NO_3^- in *S. halepense* invaded areas compared to native soils. NH_4^+ and NO_2^- metabolism acidifies soils and promotes dissolution of hydroxyapatite ultimately resulting in an increase in available P, Ca^{2+}, and Fe^{3+} for plant uptake. These latter processes are also promoted by action of endophytic pseudomonads and contribute to overall higher soil P and Fe^{3+}, and lower Ca^{2+} in invaded soils

plant–soil feedback systems that seem to be driven by plant growth-promoting bacteria and processes associated with soil microflora. A similar, but more advanced conceptual model has been proposed for the role of plant growth-promoting bacteria in mangrove ecosystems (Bashan and Holguin 2002). Novotny et al. (2007) recently reported findings that also lend support to our general model. They found that N-availability, changes in CO_2 levels, and changes in plant community diversity interact to affect both above-ground and below-ground processes. Additional research will be required to separate the

effects of root exudates from those connected to plant growth-promoting bacteria.

Many hypotheses have been put forth to explain successful plant invasions, and most focus on unusual traits of the invasive plant itself (empty niche, novel weapons, adaptation to humans) or escape from regulating consumers and pathogens (summarized by Mitchell et al. 2006). Microbial associations with plants, both bacterial and fungal, are certainly not unusual. In the case of plant invasions, associations with microbes are most often thought to have negative affects on plants rather than mutualistic relationships, accounting for the expansion of an invader when it escapes microbial pathogens. We have demonstrated that the highly invasive *S. halepense* establishes and persists in exceptionally N-poor soils and appears to do so, in part, as a consequence of a relationship with endophytic and closely associated root bacteria. This consortium of bacteria contains N_2-fixers and other plant growth-promoting bacteria that alter biogeochemical cycles in soils harboring *S. halepense*. In this system, it appears that the microbial partners form a mutualistic relationship with the invasive *S. halepense*, which we refer to as microbial enhanced competitive ability (MECA). This idea of a plant–microbial mutualism enhancing the success of an invasive plant may shed new light on how some invasives, like *S. halepense*, acquire a competitive advantage over native plant communities not accounted for by exploitation of an empty niche or by enemy release. Thus, a key component to understanding invasive plant establishment, persistence, and the cascade of ecosystem changes that follow, may reside literally within the plants themselves, as appears to be the case for *S. halepense*.

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