SUPPLEMENTAL SEED INCREASES NATIVE SEEDLING ESTABLISHMENT IN ROADSIDE PRAIRIE RESTORATION

Submission Category: Research Article

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Author contributions: JLR, LLJ conceived and designed the research; JLR performed the experiment; JLR, MES analyzed the data; JLR, MES, LLJ wrote and edited the manuscript; MES made the figures.

ABSTRACT

Tallgrass prairie restorations are plagued by high seed costs and low rates of seedling establishment. Many restorations suffer high rates of seed loss to granivores, yet to date, there are no established protocols to minimize their impact. In this study, we tested whether the application of supplemental (sacrificial) seed reduces native seed consumption and increases native seedling establishment in roadside prairie restoration. We applied supplemental birdseed to a random subset of research plots at three roadside prairie restoration sites and compared rates of seed consumption and early native seedling establishment between supplemental seed plots and control plots. All three roadside restorations were seeded in fall 2014, immediately following the first frost. To assess native seed consumption, we monitored rates of seed removal from "seed cards" during the first 14 days of the restorations. To assess early seedling establishment, we identified and counted all native seedlings in mid-July of the first restoration year. The application of supplemental seed did not reduce rates of seed consumption, which were very low during the early stages of these restorations, but did increase early native seedling establishment. Native seedling establishment was approximately 37% higher in supplemental seed plots than in control plots across restoration sites. The application of supplemental seed may have increased seedling establishment by reducing consumption of native seed during winter and spring. Our results suggest that supplemental seed is a practical, inexpensive technique for increasing seedling establishment in roadside prairie restoration.

Keywords: buffet experiment, roadside restoration, seed predation, seedling establishment, supplemental seed, tallgrass prairie restoration

IMPLICATIONS FOR PRACTICE

- This study suggests that it is possible to mitigate seed loss due to granivores and increase native seedling establishment in roadside prairie restoration by using supplemental seed.
- One mechanism through which increased seedling establishment could improve the success of roadside prairie restoration is by reducing weed biomass. High weed biomass can delay or prevent native establishment, reduce native richness and diversity, and increase management costs in prairie restoration.
- In this study, supplemental seed increased the cost of roadside prairie restoration by approximately 16% while boosting seedling establishment by approximately 37%. This suggests that practitioners may be able to reduce native seeding rates, and therefore net costs, by using supplemental seed in roadside prairie restoration.

INTRODUCTION

The tallgrass prairie ecosystem, which once covered approximately 100 million ha of North America, now occupies less than 3 percent of its original expanse (Sampson & Knopf 1994; Smith 1998; Smith et al. 2010). Restoration practitioners are attempting to reestablish this endangered ecosystem and the services it once provided, but these projects are notoriously costly (Gerla et al. 2012). A low-diversity seed mixture (20-30 species) can cost between \$500 and \$1500 ha^{-1}, while a high-diversity mixture (50-70 species) can cost as much as \$5000 ha^{-1} (Prairie Moon Nursery 2013). Low seedling establishment is a major contributor to this cost. Restoration practitioners typically sow between 400 and 950 pure live seeds (PLS) m^{-2} to achieve a final stand density of 30 adult plants m^{-2} (Smith et al. 2010; Williams 2010): an establishment rate of 3.1 – 7.5%. Identifying the causes of seed loss in native prairie restoration, and developing protocols to minimize this loss, could improve restoration success.

Small vertebrate granivores, such as meadow voles, field mice, and birds, can be a significant cause of seed loss in tallgrass prairie restorations. Previous studies have shown that these granivores can reduce seed number in prairie restorations (Howe & Brown 1999; Clark & Wilson 2003; Hemsath 2007) and alter the composition of the emerging community (Howe & Brown 1999, 2000). Further, a recent study found that small vertebrate granivores reduce native seedling emergence by approximately 30% in newly restored prairies (Pellish et al. in press). This reduction in seedling emergence could lead to higher weed establishment, which could delay or prevent native seedling establishment, increase management costs, and reduce the overall quality of the restoration (Schramm 1990; Blumenthal et al. 2003, 2005; Grman & Suding 2010; Dickson et al. 2010; Martin & Wilsey 2012; Nemec et al. 2013). To date, there are no established protocols for reducing the impact of small vertebrate granivores in native tallgrass prairie restoration.

One technique that could reduce vertebrate granivory during prairie restoration is the use of chemical feeding deterrents. Capsaicin, for example, is an effective feeding deterrent against mammalian granivores (Levey et al. 2006) and increases seedling recruitment for some prairie species during restoration (Hemsath 2007). Similarly, the fungicide Thiram emits a sulfurous odor that repels birds and deer mice from agricultural seed (Nolte & Barnett 2000; Ngowo et al. 2005). However, this technique may not be feasible for prairie restoration because of the variable morphology of prairie seeds (size, shape, and texture) and the environmental exposure that seeds experience before germination. Another technique that could reduce vertebrate granivory during prairie restoration is the application of supplemental (sacrificial) seed. The use of supplemental seed is based on the evolutionary principles of mast seeding (Janzen 1971; Kelly 1994; Kelly et al. 2000; Kelly & Sork 2002) and optimal diet theory (Sih & Christensen 2001). Mast seeding is the intermittent production of large synchronized seed crops. In high seed years, the seed crop satiates granivores, reducing overall seed loss. Optimal diet theory suggests that granivores should preferentially consume seeds that provide higher net energy intake per unit handling time (Janzen 1971; Pulliam & Brand 1975; Kerley & Erasmus 1991; Sih & Christensen 2001). Based on these principles, providing granivores with an abundant, higher-calorie seed source should reduce consumption of native seeds during restoration. If so, the application of supplemental seed could be a practical, inexpensive technique for improving restoration success.

Roadsides are an integral component of restoration efforts in Iowa, U.S.A. (Smith 1998; Houseal & Smith 2000). In 1988, Iowa legislation established the integrated roadside vegetation management (IRVM) program and the Living Roadway Trust Fund to support the restoration and protection of native vegetation in the state's roadsides (Brandt et al. 2015). Since that time, more than 20,000 ha of roadside have been restored to native prairie (Iowa Department of Transportation 2017). In addition to improving the aesthetics of Iowa's roadsides, these restoration projects reduce soil erosion, improve water quality, reduce herbicide use, and provide valuable habitat for wildlife (Christiansen & Lyons 1975; Flynn 1994; Ries et al. 2001; Brandt et al. 2015). Improving the success of these roadside restoration projects will contribute to the overall recovery of the tallgrass prairie ecosystem.

In this study, we examine the effect of supplemental seed addition on roadside prairie restoration. We applied supplemental birdseed to research plots at three roadside restoration sites and compared rates of seed consumption and native seedling establishment between supplemental seed plots and control plots. We predicted that the application of supplemental seed would reduce consumption of native seeds during the initial stages of the roadside restoration and increase early native seedling establishment.

METHODS

Study Sites

This research was conducted in 2014 and 2015 at three county roadside restoration sites in Iowa, U.S.A. One site was located in Linn County, Iowa and two sites were located in Benton County, Iowa (Fig. S1). For convenience, we refer to these as the Linn, Benton North (Benton N) and Benton South (Benton S) sites throughout the article. The roadsides were regraded in summer 2014 and seeded with native prairie vegetation in fall 2014 by the Linn and Benton County Secondary Roads Departments.

Linn - The Linn County restoration site was 0.11 ha in size and located in Marion, Iowa (42° 2' 6.6" N; 91° 31' 13" W, Fig. S1). The site was on the south side of Marion Airport Road, which runs east to west. Prior to restoration, the site was dominated by nonnative grasses, including: *Agropyron repens* L. (Quack grass), *Bromus inermis* Leyss. (Smooth brome), *Medicago sativa* L. (Alfalfa), *Poa pratensis* L. (Kentucky bluegrass), and *Trifolium pratense* L. (Red clover). Management consisted of biannual roadside mowing and spot-spraying or mowing of noxious weeds. The soil at Linn is classified as Klinger-Maxfield silty clay loams (Natural Resources Conservation Service 2013). In summer 2014, the vegetation and a layer of topsoil were stripped from the site. On August 6 2014, the site was hydroseeded with a cover crop of *Avena sativa* L. (Common oats; 5.6 g m^{-2}), *Elymus canadensis* L. (Canada wild rye; 0.11 g m^{-2}), and *Secale cereale* L. (Cereal rye; 5.6 g m^{-2}) using a Finn T-90 hydroseeder (Finn Corporation, Fairfield, OH, U.S.A.) and then cultipacked with a Reinco 6-foot mulch disc cultipacker (Reinco Inc., Fairfield, NJ, U.S.A.).

On September 26 2014, we established six research plots at Linn. The plot sizes were 37 m (east to west) × 5 m (north to south). The first frost at Linn occurred on October 30 2014. On November 12 2014, the Linn County Secondary Roads Department drill-seeded the site with a 30-species mixture (Table S1) at the rate of 1.174 g m^{-2} (576.5 seeds m^{-2}) using a Truax grass drill (Truax Company, Inc., New Hope, MN, U.S.A.). The site was mowed on June 23 2015, to manage weeds and prevent the cover crop from becoming the dominant vegetation.

Benton N and Benton S - The Benton County restoration sites were located near Atkins, Iowa (Benton N: 41° 59’ 7” N; 91° 51’ 13” W, Benton S: 41° 57’ 29” N; 91° 51’ 13” W, Fig. S1). Benton N was 0.3 ha in size and Benton S was 0.55 ha in size and both were located on the east side of 33rd Avenue / West 28th Street in a portion of the roadside that runs north to south. Prior to restoration, both sites were dominated by the nonnative grass *Bromus inermis* Leyss. (Smooth brome) and management consisted of occasional spraying with 2-4D or Milestone for noxious weeds. The soils at Benton N and Benton S are classified as Kenyon loam (Natural Resources Conservation Service 2013). In late summer 2014, the vegetation and a layer of topsoil were stripped from the site to make the ditch wider, less steep, and to create a shoulder for the road. On September 8 2014, the sites were planted with a cover crop of *Triticum aestivum* (Winter wheat; 5.6 g m^{-}). The cover crop was drill-seeded on the bottoms and foreslopes with a Truax Flex II drill (Truax Company, Inc., New Hope, MN, U.S.A.) and hydroseeded on the backslopes using a Finn hydroseeder (Finn Corporation, Fairfield, OH, U.S.A.). On September 26 2014, eight plots were established at Benton N and 15 plots were established at Benton S. Each plot was 10 m (east to west) × 37m (north to south). The first frost at the Benton sites occurred on October 23 2014. On October 29 2014, the Benton County Secondary Roads Department drill-seeded the foreslopes and bottoms of both sites with a 35-species mixture (Table S1) at a rate of 5.6 g m^{-2} (3014.9 seeds m^{-2}) using a Truax Flex II drill (Truax Company, Inc., New Hope, MN, U.S.A.). The backslopes were hydroseeded with the same seed mixture on the same day at the same seeding rate (5.6 g m^{-2}) using a Finn hydroseeder (Finn Corporation, Fairfield, OH, U.S.A.).

In spite of their close proximity and identical seeding protocol, we treated Benton N and Benton S as separate sites because of differences in ditch profile and hydrology. In particular, Benton N was a steeper, dryer, upland site, while Benton S was a flatter, wetter, lowland site.

Supplemental Seed

The supplemental seed mixture consisted of an equal proportion (by mass) of four types of birdseed: black oil sunflower (*Helianthus annuus* L.), Nyjer thistle (*Guizotia abyssinica* L. f. Cass.), white millet (*Panicum miliaceum* L.), and cracked corn (*Zea mays* L.). All seed was obtained from Cedar River Milling Company (Waterloo, IA, U.S.A.). We added mineral salt to the supplemental seed mix at the rate of 4.1 g m^{-2} to increase palatability (Weeks & Kirkpatrick 1976; Robbins 1983). To ensure that the supplemental seed would not germinate, we roasted the sunflower and millet seeds at 180°C for 30 minutes (Corbineau et al. 2002). To confirm that the seed was not viable, we attempted to germinate approximately 1 kg of roasted seed and detected no germination. The thistle seed was pre-sterilized and the corn was cracked, which renders the embryos non-viable.

We applied the supplemental seed treatment immediately after the native species were seeded (October 29 2014 at Benton N and Benton S; November 12 2014 at Linn). Supplemental seed was applied to approximately half of the research plots: three (of six) plots at Linn, four (of eight) plots at Benton N, and eight (of 15) plots at Benton S (Fig. S1). The plots receiving the supplemental seed were chosen randomly. Supplemental seed was applied at ten times the seeding rate of the native seed (56 g m^{-2} at Benton N and Benton S; 11.74 g m^{-2} at Linn) using a hand broadcast seed spreader (PlantMates model 76300, PlantMates LLC, Pasadena, TX, U.S.A.).

Seed Removal Experiment

To assess granivory during the initial stages of these restorations, we monitored the rate of seed removal from “seed cards” using a buffet-style experiment (e.g., Westerman et al. 2003; Heggenstaller et al. 2006). The seed cards consisted of 30 well-filled *Echinacea pallida* seeds glued to an 11 cm x 14 cm piece of coarse sand paper (3M Paper Sheet 346U, 36 Grit, aluminum oxide commercial D-weight, 3M Company, Maplewood, MN, U.S.A.). To attach the seeds, we applied a base layer of aerosol spray adhesive (3M Super 77 Multipurpose Adhesive Aerosol, 3M Company, Maplewood, MN, U.S.A.) to the sand paper, placed the 30 seeds on the adhesive, waited 24 hours, and then covered the seeds with another layer of aerosol spray adhesive. Previous research suggests that the adhesive and sandpaper do not attract or deter predators from the seeds (Westerman et al. 2003). We allowed the adhesive to dry for 48 hours before placing the cards in the field. Seed cards were affixed to the soil using 5.1 cm roofing nails. We chose *Echinacea pallida* because of seed morphology (relatively small, yet manageable to work with and identify for counting) and because it was in the seed mixture at each restoration site.

We conducted two seed card trials in this experiment. First, we conducted a seven-day trial before the sites were seeded ("pre-planting trial": September 26 – October 3 2014) to assess background levels of granivory at each site. All remaining seeds were counted on the seventh day of this trial (October 3 2014) to quantify seed loss. Any seed that was missing, chewed, or broken was considered consumed. Second, we conducted a 14-day trial after the sites were seeded ("post-planting trial": October 29 – November 12 2014 at Benton N and Benton S; November 12 – 26 2014 at Linn). Remaining seeds were counted on the seventh and 14th day of this trial to quantify seed loss. We used the same protocol in the pre- and post-planting trials. Specifically, we placed seven seed cards, equidistant to one another, along a transect at the center of each plot (Fig. S2). To assess passive seed loss to factors such as wind, rain, adhesive failure or flaws in card design, we placed a control seed card next to one, randomly-selected seed card in each plot. The control seed card was placed inside a metal cage (32 cm x 14 cm x 8 cm) and surrounded by insect barrier cloth (Agribon + AG 15, 118'' X 50', lightweight grade) to exclude small vertebrate granivores and invertebrate granivores respectively. We compared rates of seed loss between the 7-day pre-planting trial and the seventh day of the post-planting trial. In the post-planting trial, we compared rates of seed loss between supplemental seed plots and control plots.

Seedling Establishment Experiment

We identified and counted all established seedlings at each roadside restoration site on July 20 - 24 2015. Because two seeding techniques were used at the Benton sites (drill seeding on foreslopes and bottoms, hydroseeding on backslopes), we divided the ditch into three sections (foreslope, bottom, backslope; Fig. S2) at each site and quantified seedling establishment within each section. The bottoms were defined as having a 0-5 degree slope, while the foreslopes (the section next to the road) and backslopes were defined as having a slope equal to or greater than five degrees as determined by a clinometer. In each plot, we identified and counted all seedlings in five, 0.1-m^2 quadrats in each section of ditch profile (i.e., 15 quadrats per plot). To minimize variation and provide a buffer between adjacent plots, all sampling was localized in the central five meters (of the long axis) of each plot (Fig. S2). Within that five-meter sampling area, quadrats were placed at five random positions along a transect at the center (by width) of each ditch section (Fig. S2). All native seedlings from the planted seed mixtures were counted and identified; however, we pooled the two *Liatris* species and the two *Carex* species to avoid misidentification of similar species (Natural Resources Conservation Service 2006; Williams 2010). Any identified seedlings that were not in the seed mixture were counted as weeds.

Data Analysis

In the seed removal experiment, we counted the remaining seeds on each seed card and quantified seed loss for each plot as the seven-card average. Less than one percent of seeds were lost from control cards, and consequently, we did not correct the data for passive seed loss. We analyzed the seed removal data using two different general linear models. First, we compared seed loss between the seven-day pre-planting trial and the first seven days of the post-planting trial in a model that had trial (pre-planting vs. post-planting) and site as fixed factors. For this analysis, we only used data from control plots in the post-planting trial because of the potential confounding effect of the supplemental seed addition. Second, we compared seed loss between supplemental seed plots and control plots at the end of the 14-day post-planting trial in a model that had treatment (supplemental seed plots vs. control plots) and site as fixed factors. The treatment × site term was removed from both models because of non-significance. Removal of this term did not alter the significance of any factor in either model.

In the seedling establishment experiment, we counted the total number of established native seedlings in the five 0.1-m^2 quadrats and divided by 0.5 to compute the number of established native seedlings per m^2 in each section of the ditch profile. To test whether the application of supplemental seed affected seedling establishment, we used a general linear model with treatment, site, and section of ditch profile as fixed factors.

In both the seed removal experiment and seedling establishment experiment, we inspected the data for normality using boxplots and scatter plots (qqnorm plots) of model residuals-versus-predicted values. Data from the seed removal experiment did not require transformation. Data from the seedling establishment experiment was cube-root transformed to improve normality. All data were analyzed in R (v. 3.1-109, R Core Team 2013).

RESULTS

Seed Removal Experiment

Granivores consumed significantly more seed in the seven-day pre-planting trial (24.8%) than in the first seven days of the post-planting trial (4.3%, F=26.15, p<0.001, Fig. 1, Table S2). In the 14-day post-planting trial, granivores only consumed 7.4% of seeds and seed consumption did not differ significantly between supplemental seed plots and control plots (F=1.35, p=0.256, Fig. 1, Table S3).

Seedling Establishment Experiment

On average, 128.4 ± 12.9 native seedlings m^{-2} (mean ± SE) established at the three roadside restoration sites (Fig. 2). Seedling establishment differed between sites (F=3.52, p=0.035, Table S4) and was significantly higher at Benton N (184.2 ± 32.3) than at Linn (103.6 ± 29.4). Seedling establishment at Benton S (107.2 ± 11.0) did not differ significantly from either of the other two sites. Because the seeding rate was lower at Linn than at the Benton sites, the seedling establishment rate was higher at Linn (18%) than at Benton N (6.1%) and Benton S (3.6%). The seedling establishment rates of each species are summarized in Table S5.

Seedling establishment was significantly higher in supplemental seed plots (157.3 ± 19.3) than in control plots (99.6 ± 16.0) across roadside restoration sites (F=13.11, p<0.001, Fig. 2, Table S4). Seedling establishment was higher in supplemental seed plots than in control plots at each individual site; however, this difference was only significant at Linn (Fig. 2).

Seedling establishment differed significantly between sections of the ditch profile across restoration sites (*F*=10.32, *p*<0.001, Table S4, Fig. 3). Seedling establishment was significantly higher on the foreslopes (196.1 ± 25.5 seedlings m^{-2}) than on the bottoms (87.9 ± 15.3 seedlings m^{-2}) and backslopes (101.3 ± 19.6 seedlings m^{-2}). On the backslopes, seedling establishment was marginally higher in supplemental seed plots than in control plots (*p*=0.07; Fig. 3). Conversely, supplemental seed addition did not affect seedling establishment on the foreslopes or bottoms (Fig. 3). There was a marginally significant site × section of ditch profile term (*F*=2.41, *p*=0.057, Table S4), which may have occurred because seedling establishment on the foreslopes and bottoms was highest at Benton N while seedling establishment on the backslopes was highest at Linn (Fig. 3). Seedling establishment was higher in supplemental seed plots than in control plots in every site × section of ditch profile treatment combination; however, this difference was never statistically significant (Fig. 3).

DISCUSSION

In this study, we tested whether the application of supplemental seed influences the success of roadside prairie restoration. To do this, we compared rates of native seed consumption (seed removal experiment) and native seedling establishment (seedling establishment experiment) between supplemental seed plots and control plots at three newly restored roadside sites. In the seed removal experiment, we found that the rate of seed loss was very low during the initial stages of these restorations and was not influenced by the supplemental seed addition. In the seedling establishment experiment however, we found that seedling establishment was approximately 37% higher in supplemental seed plots than in control plots across restoration sites. Collectively, our results suggest that the application of supplemental seed increases native seedling establishment in roadside prairie restoration and that this may be due to lower consumption of native seed during winter and spring.

Supplemental seed may have increased seedling establishment in roadside restorations by reducing consumption of native seed. Two mechanisms through which supplemental seed could reduce native seed consumption are predator satiation and predator manipulation. Predator satiation is related to seed density. At higher seed densities, granivores consume a lower proportion of available seed (e.g., Cardina et al. 1996; Edwards & Crawley 1999), thus leaving more native seeds to germinate and become established. This interpretation is consistent with the evolutionary principle of mast seeding as a defense against granivores (Janzen 1971; Kelly 1994). Predator manipulation is related to optimal diet theory. Preferential consumption of the more abundant, higher-calorie birdseed may have resulted in lower consumption of the less abundant, lower-calorie native seed (Janzen 1971; Kerley & Erasmus 1991; Sih & Christensen 2001). These two mechanisms are not mutually exclusive and could be operating in combination.

The effect of supplemental seed on seedling establishment was remarkably consistent in our study, suggesting that this technique may improve the success of a wide variety of roadside restoration projects. The three restoration sites used in our study differed in profile and hydrology, and were restored using different seed mixtures, seeding rates, and seeding methods. In spite of this variability, native seedling establishment was always higher in supplemental seed plots than control plots (at every site, in every section of the ditch profile, and in every site × section of ditch profile treatment combination) although the effect was not always significant. Previous studies have shown that granivores are an important cause of seed loss in prairie restorations and significantly reduce native seedling emergence (e.g., Howe & Brown 1999; 2000; Clark & Wilson 2003; Pellish et al. 2017). Our study supports these conclusions and suggests that the application of supplemental seed is an effective technique for reducing this impact and improving the success of roadside restoration projects.

Differences in seedling establishment between sections of the ditch profile could be related to soil moisture. Seedlings on the roadside bottoms had to endure periods of standing water, which may have lead to higher mortality and lower establishment in this section. On the foreslopes and backslopes, soil moisture may have been influenced by aspect (the direction a slope faces). In the Northern hemisphere, north- and east-facing slopes are relatively wetter than south- and west-facing slopes at the same elevation. This can lead to higher establishment on north- and east-facing slopes in roadside restorations (Bochet & García-Fayos 2004; Bochet et al. 2007). We found mixed support for this interpretation. Seedling establishment was indeed higher on the east-facing foreslope than the west-facing backslope at Benton N (ANOVA: $F = 12.503$, $p = 0.004$) and marginally higher on the east-facing foreslope than the west-facing backslope at Benton S (ANOVA: $F = 3.251$, $p = 0.083$); but seedling establishment was not higher on the north-facing backslope than the south-facing foreslope at Linn (ANOVA: $F = 0.195$, $p = 0.703$). In spite of these overall differences in seedling establishment, the influence of supplemental seed on seedling establishment did not differ between sections (non-significant treatment × section term: $F = 0.58$, $p = 0.58$, Table S4). This suggests that practitioners should apply supplemental seed evenly to all sections of the ditch profile, regardless of differences in soil moisture.

In contrast to our prediction, the application of supplemental seed did not reduce native seed consumption during the initial stages of roadside restoration. Our seed removal experimental did however highlight temporal variation in rates of seed predation. Seed predation varies temporally based on consumer presence and activity (Howe & Brown 2000; Heggenstaller et al. 2006). In our study, we detected significantly higher seed removal in the seven-day pre-planting trial, which occurred before the first frost, than the first seven days of the post-planting trial, which occurred after the first frost. These results suggest that invertebrates, which would have been present in the pre-planting trial and absent from the post-planting trial, are an important cause of seed loss in roadside restoration. The impact of invertebrate granivores on prairie restoration is poorly understood, but previous studies have shown that invertebrates are an important cause of seed loss for some prairie and weed species in the Midwestern United States (Clark & Wilson 2003; Gaines & Gratton 2010). In contrast to invertebrates, mammalian granivores may have avoided these roadside restoration sites because there was low vegetative cover in the roadsides and surrounding agricultural fields (Heggenstaller et al. 2006; Baraibar et al. 2009). Rodents can be important consumers of prairie seed during winter (Westerman et al. 2008), when seed loss was not being monitored in our study. Future studies should attempt to quantify seed loss throughout the restoration process to better understand peak predation times for different granivores. This data would help practitioners determine the optimum time to apply supplemental seed. Although seed loss was low during the initial stages of these restoration, consumption of native seed was likely higher in control plots than supplemental seed plots at some point during the restoration process because of the ultimate difference in seedling establishment between treatments.

Management Implications

One way that supplemental seed could improve the outcome of roadside restoration projects is by reducing weed establishment. Restorations with high native establishment typically have fewer weeds (Blumenthal et al. 2003, 2005; Middleton et al. 2010; Carter & Blair 2012; Nemec et al. 2013). The establishment of weeds can delay or even prevent native seedling establishment, and ultimately reduce native richness and diversity in prairie restorations (Blumenthal et al. 2003; Martin and Wilsey 2012). High weed biomass can also increase the management costs ofprairie restoration. Another way that supplemental seed could improve roadside restoration success is by reducing granivore-induced effects on community composition. Granivores can alter community composition by preferentially consuming certain species (Howe & Brown 1999). The presence of high-calorie birdseed may reduce the likelihood that native species with relatively high-calorie seeds will be preferentially consumed and thereby increase the overall richness and diversity of a restoration.

Because seed costs are already a prohibitive aspect of prairie restoration, it is important to consider the cost and benefits of supplemental seed. Using the native seed mixture (IRVM Diversity Mix) and seeding rates (prairie seed = 1.174 g m^{-2}, supplemental seed = 11.74 g m^{-2}) at Linn as an example, the cost of native seed was \$811 ha^{-1} (personal communication, Kristine Nemec, University of Northern Iowa) and the cost of supplemental seed was \$129 ha^{-1}. Therefore, supplemental seed increased the total seed cost of the restoration project by approximately 16% (\$940 ha^{-1}). Practitioners may be able to offset this additional cost by reducing native seeding rates. The application of supplemental seed increased native seedling establishment by 37%. If restoration practitioners could achieve the same native seedling establishment with 37% fewer native seeds, it would reduce the total seed cost to \$640 ha^{-1} (prairie seed: \$511 ha^{-1}; supplemental seed: \$129 ha^{-1}): a 21% reduction in seed cost. It should be noted however, that an increase or decrease in native seeding rates does not always correspond to a proportional increase or decrease in native seedling establishment (Williams and Smith 2007). Future research should examine different combinations of native and supplemental seeding rates to determine the optimum combination from an economic perspective.

Roadside restoration projects are an important component of restoration efforts in Iowa, U.S.A. (Smith 1998; Houseal & Smith 2000) and contribute to the overall recovery of the tallgrass prairie ecosystem. There are several unique challenges associated with roadside restoration projects, including: slope, aspect, disturbance (e.g., gravel deposition via snowplow), and environmental heterogeneity (e.g., soil moisture, light and nutrient availability, soil type). Our study suggests that supplemental seed significantly increases native seedling establishment during the critical early stages of roadside restoration projects. This technique could help practitioners overcome the challenges of roadside restoration.

ACKNOWLEDGEMENTS

We thank J. Abernathy, K. Elgersma, G. Houseal, K. Nemec, D. Williams, and J. Young for helpful comments and discussions on the manuscript. We thank R. Roman and B. Bonar for seeding and managing the roadside sites. We thank A. Andrews, J. Cubit, W. Pingel, O. Schouten, C. Swanson, and D. Williams for assistance in the field. We thank D. Williams for assistance with seedling identification. This work was supported by the Living Roadway Trust Fund and the University of Northern Iowa.

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SUPPORTING INFORMATION

Figure S1. Locations and plot layouts of the three roadside restoration sites.

Figure S2. Photo of roadside (highlighting sections) and schematic diagram of plots.

Table S1. Seed mixtures and seeding rates for the Linn and Benton County sites.

Table S2. General linear model: seed removal experiment (trial effect).

Table S3. General linear model: seed removal experiment (treatment effect).

Table S4. General linear model: seedling establishment experiment.

Table S5. Average number of established seedlings m^{-2} of each species at each site.

FIGURE CAPTIONS:

Figure 1. Seed consumption at three roadside prairie restoration sites. Seed consumption was quantified for each plot as the average number of seeds removed from seven seeds card after seven or 14 days. We present the data as the average of all plots (±1SE) at all three sites. Seed consumption was significantly higher in the seven-day pre-planting trial (grey triangles) than in the first seven days of the post-planting trial. For this analysis, we only used data from control plots in the post-planting trial (grey circles) because of the potential confounding effect of supplemental seed addition. Seed consumption did not differ between supplemental seed plots (white circles) and control plots (grey circles) in the 14-day post-planting trial.

Figure 2. The number of established seedlings per m^{-2} in supplemental seed plots (white bars) and control ploys (grey bars) at three roadside restoration sites, as well as the three-site average. Data presented are means ±1SE. Significant differences between treatments, based on Tukey post hoc tests, are indicated with asterisks. Dashed lines represent site averages (across treatments) and significant differences between sites are indicated with different letters.

Figure 3. The number of established seedlings per m^{-2} in supplemental seed plots (white bars) and control ploys (grey bars) in each section of the ditch profile at three roadside restoration sites, as well as the three-site average. Data presented are means ±1SE. Significant differences between treatments, based on Tukey post hoc tests, are indicated with asterisks (° indicates a marginally significant [$p < 0.10$] difference). Dashed lines represent section averages (across restoration sites).

Figure 1.

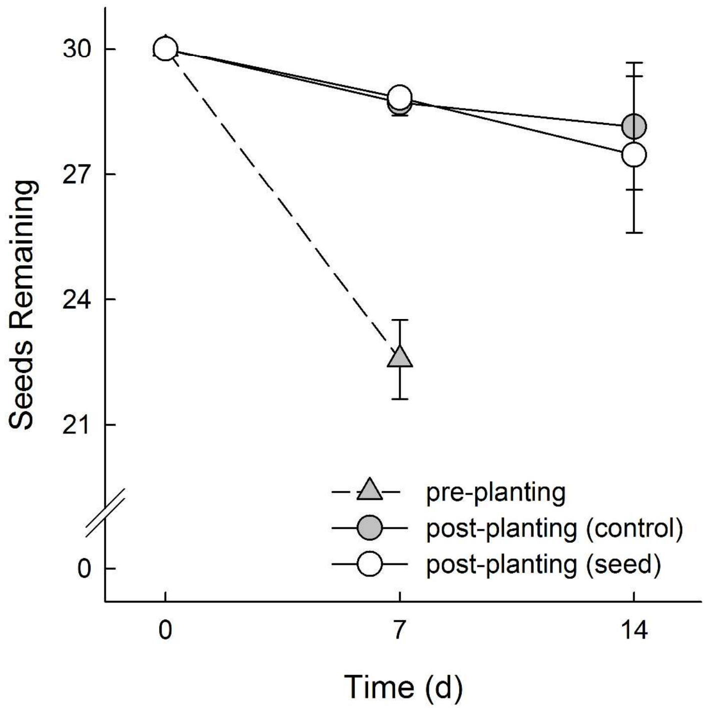

Seeds Remaining
30
27
24
21
0
0
7
14
Time (d)
pre-planting
post-planting (control)
post-planting (seed)

Figure 2.

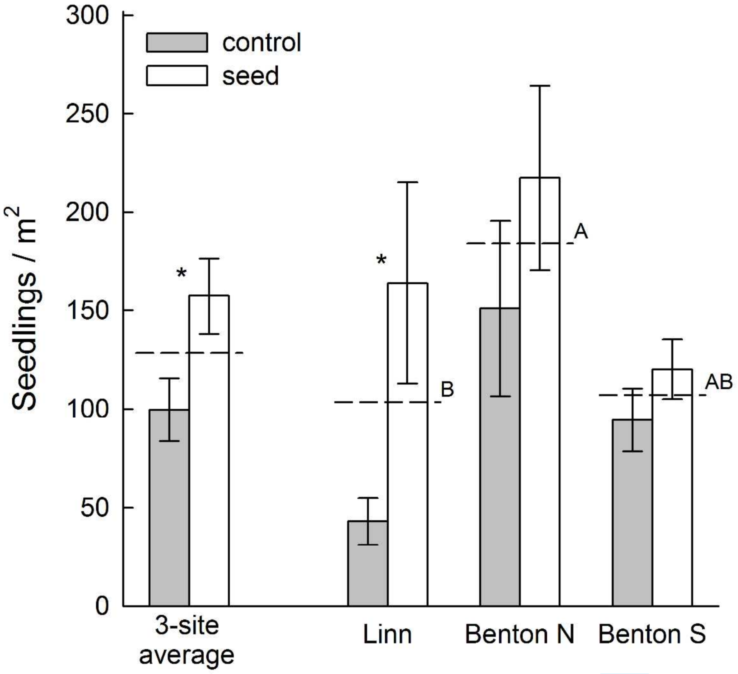
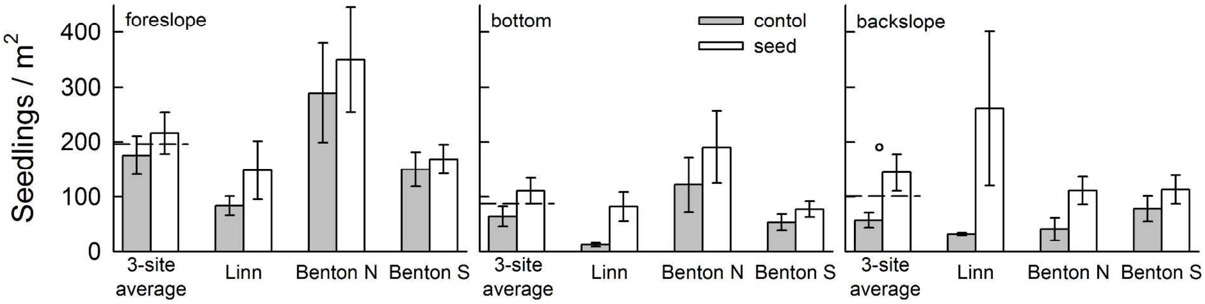

300
250
200
150
100
50
0
control
seed
Seedlings / m^2
*
*
A
B
AB
3-site average
Linn
Benton N
Benton S

Figure 3.


foreslope
bottom
backslope
contol
seed
Seedlings / m^2
400
300
200
100
0
3-site average
Linn
Benton N
Benton S
°

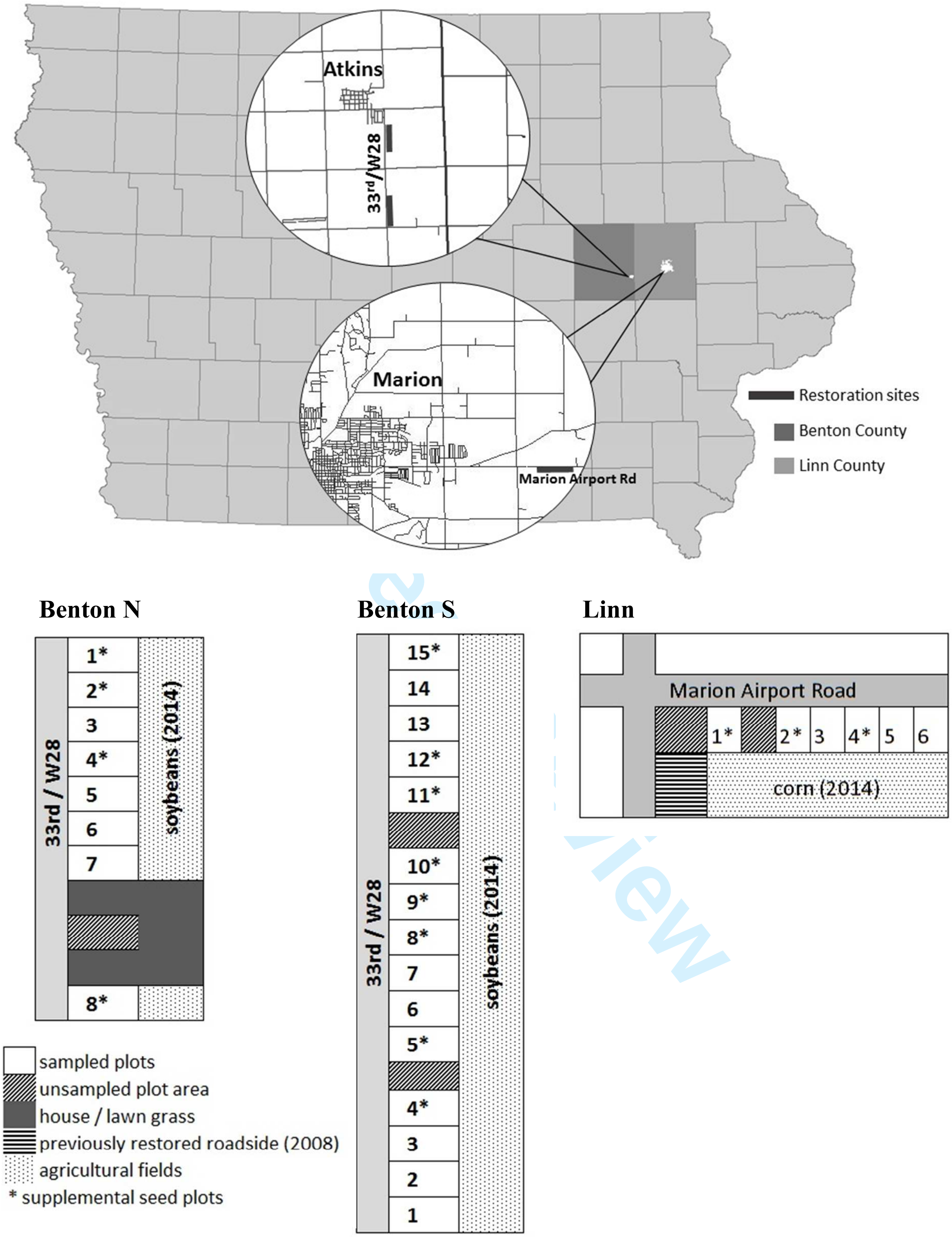


Figure S1. Above: County map of Iowa highlighting the location of the three restoration sites in Benton and Linn Counties. Below: Plot layout at the three restoration sites: Benton N, Benton S, and Linn.

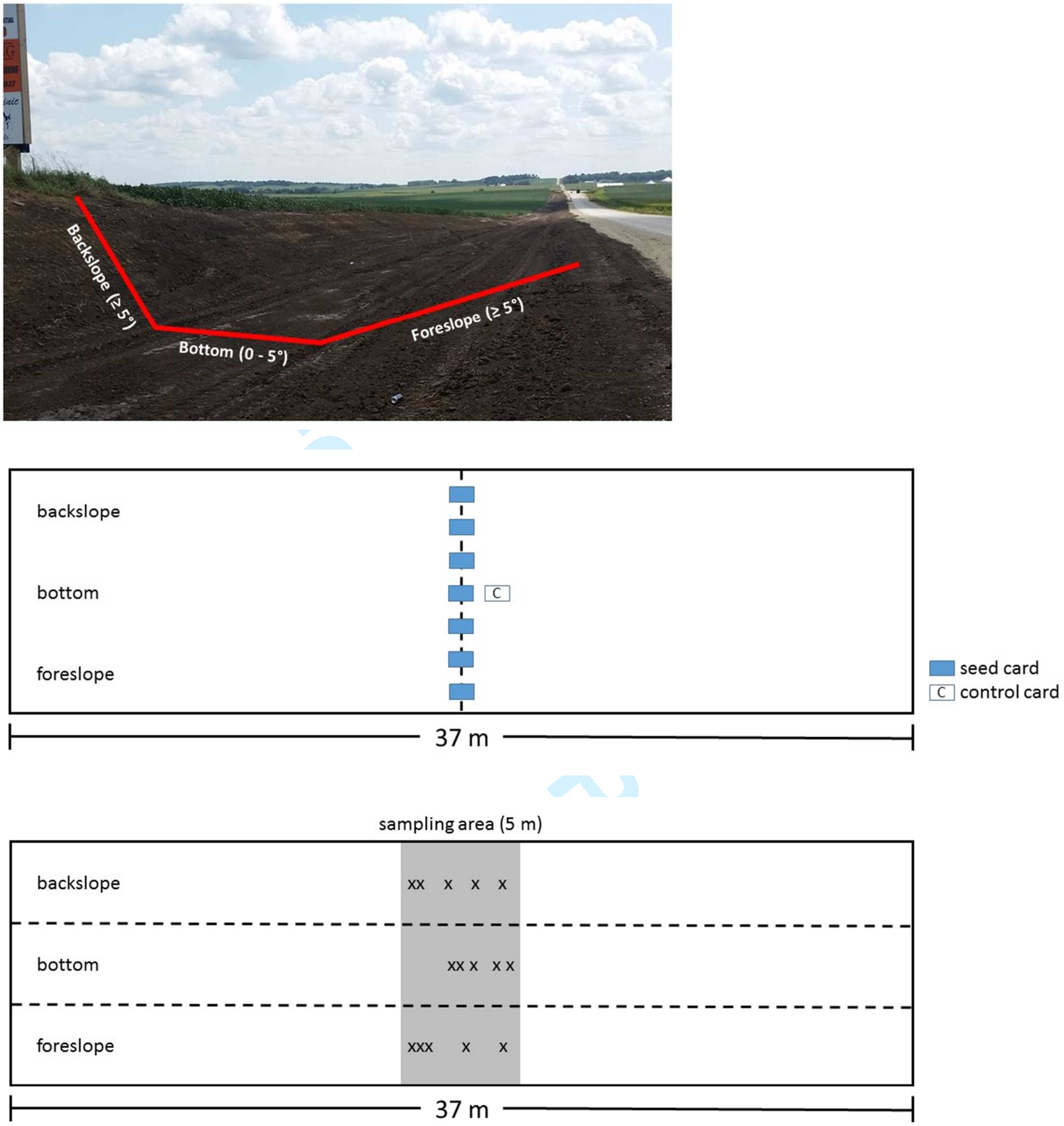


Figure S2. Above: Photograph of a roadside restoration site, highlighting the three sections of the ditch profile. Center: Schematic diagram of one research plot in the seed card experiment. Seed cards were placed equidistant to one another (spanning all three sections of the ditch profile) along a transect located the center of the longest plot axis. A control card was placed next to one randomly selected seed card in each plot. Below: Schematic diagram of one research plot in the seedling establishment experiment. Sampling occurred in a 5 m strip located at the center of the longest plot axis, creating a 16 m buffer between the sampling area and adjacent plot on both sides. Five 0.1-m quadrats (represented by Xs) were randomly placed along a transect located at the center (by width) of each ditch section.

Table S1. Seed mixtures for the Linn and Benton County sites. Species are listed alphabetically and nomenclature is based on USDA plants database (USDA-NRCS 2015).

	Linn		Benton	
	g m^{-2}	seeds m^{-2}	g m^{-2}	seeds m^{-2}
Amorpha canascens	0.02	9.88	0.07	42.07
Andropogan gerardii Vitman	0.13	47.44	0.57	201.72
Asclepias incarnata L.	.	.	0.09	15.13
Asclepias tuberosa L.	0.02	2.66	0.07	11.29
Astragalus canadensis L.	0.02	10.08	0.07	42.87
Baptisia alba (L.) Vent.	0.01	0.84	0.06	3.57
Bouteloua curtipendula (Michx.) Torr.	0.13	28.47	0.57	121.03
Carex bicknellii Britton	0.00	11.68	0.01	49.64
Carex vulpinoidea Michx.	.	.	0.01	31.52
Chamaecrista fasciculata (Michx.) Greene	0.12	7.41	0.52	31.52
Dalea purpurea Vent.	0.02	12.97	0.10	55.16
Desmodium canadense (L.) DC.	0.01	1.63	0.04	6.93
Echinacea pallida Nutt.	0.02	4.50	0.10	19.12
Elymus canadensis L.	0.11	20.56	0.48	87.41
Eryngium yuccifolium Michx.	0.02	5.56	0.09	23.64
Helenium autumnale L.	.	.	0.03	136.58
Heliopsis helianthoides (L.) Sweet	0.03	7.01	0.13	29.79
Lespedeza capitata Michx.	0.01	3.95	0.06	16.81
Liatris aspera Michx.	0.02	13.84	0.10	58.83
Liatris pycnostachya Michx.	0.03	11.42	0.13	48.54
Monarda fistulosa L.	0.02	43.24	0.07	183.86
Oligoneuron rigidum (L.) Small	0.01	12.16	0.04	51.69
Panicum virgatum L.	.	.	0.48	235.34
Penstemon grandiflorus Nutt.	0.01	5.19	0.04	22.06
Ratibida pinnata (Vent.) Barnhart	0.02	25.95	0.10	110.31
Rudbeckia hirta L.	0.02	56.83	0.07	241.64
Ruellia humilis Nutt.	0.01	1.54	0.04	6.56
Scirpus atrovirens Willd.	.	.	0.01	144.99
Silphium lacinatum L.	0.01	0.20	0.04	0.83
Sorghastrum nutans (L.) Nash	0.13	56.93	0.57	242.06
Sporobolus compositus (Poir.) *Merr.*	0.13	132.84	0.57	564.81
Symphyotrichum novae-angliae (L.) G.L. Nesom	0.01	19.57	0.04	83.21
Tradescantia ohiensis Raf.	0.03	7.91	0.12	33.62
Verbena stricta Vent.	0.01	8.30	0.04	35.30
Zizia aurea (L.) W.D.J. Koch	0.02	5.98	0.07	25.42
	1.17	576.54	5.61	3014.88

Table S2. General linear model comparing seed loss between the seven-day pre-planting trial and the first seven days of the post-planting trial. We only used data from the control plots of the post-planting trial because of the potential confounding effect of supplemental seed addition. Trial (pre-planting vs. post-planting) and site were fixed factors in the model. The treatment × site interaction term was removed from the model because it was not significant. Removal of this term did not alter the significance of any factor. Data presented are degrees of freedom (*df*), sum of squares (SS), mean squares (MS), *F*-statistics (*F*), and *P*-values (*p*). Significant terms are indicated in bold.

Factor	*df*	SS	MS	*F*	*p*
trial	1	378.20	378.20	26.15	**<0.001**
site	2	6.81	3.41	0.24	0.792
residuals	23	332.64	14.46		

Table S3. General linear model comparing seed loss between treatments (control plots vs. supplemental seed plots) and sites in the 14-day post-planting trial. Treatment and site were fixed factors in the model. The treatment × site interaction term was removed from the model because it was not significant. Removal of this term did not alter the significance of any factor. Data presented are degrees of freedom (*df*), sum of squares (SS), mean squares (MS), *F*-statistics (*F*), and *P*-values (*p*). Significant terms are indicated in bold.

Factor	*df*	SS	MS	*F*	*p*
treatment	1	3.28	3.28	1.35	0.256
site	2	18.55	9.28	3.82	**0.036**
residuals	25	60.65	2.43		

Table S4. General linear model comparing the number of established seedlings between roadside restoration sites (site), treatments (control plots vs. supplemental seed plots), sections of the ditch profile (section) and their interactions. Site, treatment, and section were all fixed factors in the model. Data were cube-root transformed to improve normality. Data presented are degrees of freedom (*df*), sum of squares (SS), mean squares (MS), *F*-statistics (*F*), and *P*-values (*p*). Significant terms are indicated in bold.

Factor	*df*	SS	MS	*F*	*p*
site	2	6.809	3.405	3.52	**0.035**
treatment	1	12.676	12.676	13.11	**<0.001**
section	2	19.953	9.977	10.32	**<0.001**
site × treatment	2	2.842	1.421	1.47	0.24
treatment × section	2	1.112	0.556	0.58	0.58
site × section	4	9.323	2.331	2.41	0.057
site × treatment × section	4	1.202	0.301	0.31	0.87
residuals	66	63.821	0.967		

Table S5: Average number of established seedlings per m^2 of each species at each roadside restoration site and at all three sites combined (overall). Data presented are means and standard errors (in parentheses). Species are ordered from most to least common across sites.

	Overall	Linn	Benton N	Benton S
Rudbeckia hirta	18.18 (2.70)	27.67 (4.45)	24.13 (4.48)	10.71 (1.96)
Echinacea pallida	11.79 (1.30)	4.67 (0.87)	14.50 (2.83)	13.29 (1.71)
Sorghastrum nutans	9.79 (2.04)	4.44 (0.70)	21.17 (6.11)	5.57 (1.40)
Verbena stricta	9.52 (1.31)	4.33 (0.59)	13.25 (3.02)	9.62 (1.80)
Heliopsis helianthoides	7.79 (0.79)	4.22 (0.69)	10.50 (1.89)	7.76 (0.87)
Penstemon grandiflorus	7.71 (1.16)	1.33 (0.41)	10.75 (2.54)	8.71 (1.62)
Ratibida pinnata	7.00 (1.29)	13.22 (1.53)	16.25 (2.97)	6.67 (1.07)
Andropogan gerardii	6.79 (1.22)	3.89 (0.78)	13.42 (3.07)	4.24 (1.27)
Eryngium yuccifolium	6.36 (0.83)	1.22 (0.20)	9.00 (2.27)	7.05 (0.85)
Bouteloua curtipendula	6.24 (1.01)	4.67 (0.79)	7.58 (1.94)	6.14 (1.54)
Zizia aurea	5.05 (0.61)	5.67 (0.59)	4.58 (1.14)	5.05 (0.88)
Ruellia humilis	3.29 (0.46)	0.44 (0.12)	4.75 (1.15)	3.67 (0.56)
Sporobolus compositus	3.02 (0.87)	8.44 (1.69)	1.83 (0.60)	1.38 (0.46)
Carex sp.	2.86 (1.18)	3.00 (0.49)	7.50 (3.95)	0.14 (0.08)
Monarda fistulosa	2.57 (0.47)	1.78 (0.40)	3.58 (1.17)	2.33 (0.55)
Tradescantia ohiensis	2.38 (0.41)	0.00 (0.00)	1.58 (0.40)	3.86 (0.71)
Desmodium canadense	2.24 (0.32)	1.22 (0.19)	3.17 (0.81)	2.14 (0.38)
Elymus canadensis	2.24 (0.47)	4.89 (0.84)	1.50 (0.35)	1.52 (0.42)
Dalea purpurea	2.14 (0.43)	0.89 (0.20)	4.92 (1.19)	1.10 (0.34)
Liatris sp.	1.60 (0.30)	1.00 (0.29)	1.58 (0.71)	1.86 (0.36)
Lespedeza capitata	1.50 (0.27)	1.33 (0.21)	2.33 (0.72)	1.10 (0.28)
Asclepias incarnata	1.10 (0.22)	.	1.92 (0.58)	1.10 (0.24)
Oligoneuron rigidum	0.76 (0.15)	1.00 (0.13)	0.92 (0.38)	0.57 (0.18)
Asclepias tuberosa	0.69 (0.18)	0.89 (0.15)	0.75 (0.43)	0.57 (0.22)
Chamaecrista fasciculata	0.67 (0.16)	2.00 (0.21)	0.67 (0.31)	0.10 (0.07)
Astragalus canadensis	0.52 (0.14)	0.67 (0.15)	0.92 (0.38)	0.24 (0.10)
Baptisia alba	0.31 (0.10)	0.00 (0.00)	0.58 (0.28)	0.29 (0.13)
Amorpha canascens	0.21 (0.11)	0.44 (0.21)	0.17 (0.12)	0.14 (0.08)
Panicum virgatum	0.14 (0.08)	.	0.25 (0.14)	0.14 (0.14)
Helenium autumnale	0.10 (0.05)	.	0.17 (0.12)	0.10 (0.07)
Symphyotrichum novae-angliae	0.05 (0.03)	0.11 (0.05)	0.00 (0.00)	0.05 (0.05)
Scirpus atrovirens	0.02 (0.02)	.	0.00 (0.00)	0.05 (0.05)
Silphium lacinatum	0.02 (0.02)	0.11 (0.05)	0.00 (0.00)	0.00 (0.00)