# Studies on the Ecology of Roadside Turfgrass Mixtures in Minnesota

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# ABSTRACT

Establishing persistent vegetation along roadsides is challenging in cold climates. These areas are subject to snowplow damage, winter freezing and ice encasement, excessive heat and drought, application of deicing salt, and poor maintenance; they also often contain poor soils. The result is low vegetation cover which leads to more soil erosion and greater vulnerability to weed invasion. We wanted to identify adapted turfgrass mixtures and characteristics or disturbances of a site that leads to poor coverage over time.

All experiments were conducted at 14 roadside research sites in Minnesota. Each of these sites was located along a two to four lane road with different traffic volumes. We collected soil for a seed bank analysis that took place in the greenhouse using the soil emergent method. Then, at these same roadsides a turfgrass mixture experiment was seeded that consisted of 40 treatments composed of monocultures, two-way mixtures, some three-way mixtures, and a single six-way mixture, in addition to four currently recommended Department of Transportation seed mixtures. Each site contained three blocks in a randomized complete block design; seven of the sites were seeded in the fall of 2018 and seven in the fall of 2019. The total turfgrass coverage was assessed at each site twice per year using the quadrat-grid intersection method.

In the first experiment, we wanted to characterize the seed bank at these different sites and understand if it affects the weed coverage over time in the field plots. We found that there were differences in the seed bank at many sites. A range of more than 9 times was found in seedling density between sites (23-209 seedlings $L^{-1}$). Differences were also found in observed species density (8-16 species $L^{-1}$) and Chao estimated species density (9-32 species $L^{-1}$) for each site. Despite the significant differences in the type and density of the seed banks, its impact was relatively low on weed coverage over time. Weed coverage was found to be lower when turfgrass coverage was maintained over time.

The next experiment sought to identify the effect of including greater turfgrass species richness in a seed mixture on the coverage over time. We found a significant positive interaction with turfgrass coverage as a function of the number of species and time (Est=0.08, S.E.=0.02, $p<0.001$). This suggests that turfgrass coverage is increasing through time when more species are included in a mixture. This finding shows that roadsides maintained without regular fertilizer applications and no supplemental irrigation after establishment would benefit from greater species richness in a seed mixture.

Finally, we wanted to identify different seeding clusters for the state of Minnesota, because the Minnesota Department of Transportation is currently recommending statewide turfgrass mixtures. If specific clusters are identified it could improve the applicability of turfgrass recommendations, and likely result in more turfgrass coverage over time. We collected soil and weather variables from each site and performed an agglomerative hierarchical cluster analysis. We validated the results of the clustering by comparing the species composition at sites. Our results suggested an optimal clustering would consist of two geographical seeding clusters in Minnesota (north and central/south) and one non-geographical cluster for sites that contain poor soil quality. A poor soil quality site generally contained more sand, greater bulk density, a higher saturated paste extract electrical conductivity, and lower organic matter. We recommend more soil testing procedures for practitioners before seeding a site. Seeding mixtures designed for these clusters in Minnesota will result in improved coverage over time allowing roadside vegetation to fulfill its intended functions.

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# Chapter 1

# A Review of Turfgrass Seed Mixtures in Cold Climates with an Emphasis on Roadsides

## Introduction

Mixtures of turfgrass species are common in areas that are regularly mowed. A common assumption is that one or only a few grass species are growing in an area, yet there are commonly many grass species: a small area of land could easily contain up to 100 species and more. Other species could consist of sedges (*Cyperaceae* spp.), rushes (*Juncaceae* spp.), legumes (*Fabaceae* spp.), and other forbs existing in a complex assemblage. One only needs to go on a walk and observe the natural environment to see this. Monocultures and other areas consisting of few species do, however, exist in new seedings; areas maintained with high fertility, regular herbicide application, and lower mowing heights (<7.6 cm); and in some pasture settings (Larson et al., 2017). This review covers the purpose, function, and benefits of seeding turfgrass mixtures, potential disadvantages, general factors influencing the results of mixture experiments, limitations of many of these experiments, results of roadside and non-roadside mixture experiments, mixing cool and warm-season turfgrasses in a cold climate, mixing turfgrass species with other functional groups, and designing turfgrass mixtures.

## Purpose and functions of turfgrass for roadsides

Seeding turfgrass mixtures on roadsides began with the advent of the interstate system in the late 1920s and was further modeled off the Autobahn in Germany (Weingroff, 2013). Using turfgrass along roadsides maintains visibility for drivers, can be relatively cost efficient to establish and maintain, reduce erosion, and provide for an aesthetically uniform landscape (Boeker, 1970; Duell & Schmit, 1975; Hottenstein, 1969; White &

Smithberg, 1972). Mixtures are seeded based on the assumption that species are differentially adapted to environmental conditions. What is often debated is how many species are needed for roadside vegetation to sufficiently fulfill these intended functions.

The intended functions of a roadside differ from non-roadside areas in several ways. Non-roadside areas that are seeded as turfgrass are usually home lawns or parks. In general, managers of parks and home lawns generally have lower tolerance for weeds and taller vegetation compared to nearby roadside vegetation. Desired characteristics for parks and home lawns usually includes a uniform plush green turf, usability for lounging or athletics, and as a potential resource for pollinators (Lane et al., 2019). For a review of cool-season roadside turfgrass management and species adaptation, see Friell & Watkins (2020).

## Benefits of turfgrass mixtures

The use of species mixtures, compared to monocultures, has been shown to have multiple benefits, including more coverage of the seeded species (Tyser et al., 1998), less weed coverage (McKernan et al., 2001), reduced disease frequency and severity (Dunn et al., 2002; Xiang et al., 2019), and extended green color (P. G. Johnson, 2003). Turfgrass mixtures also have the potential to fulfill more functions (Hector & Bagchi, 2007) sometimes in unseen ways. For instance, turfgrass species may have different rooting depth and heterogeneity (Brown et al., 2010) and a mixture designed with this function can reduce erosion (Simon & Collison, 2002). Burt et al. (2020) suggested that planting a mixture of 27 species, including turfgrasses, could potentially support up to 520 insect species. Xie et al. (2020) found a mixture of strong creeping red fescue (*Festuca rubra* L. ssp. *rubra* Gaudin) and Kentucky bluegrass (*Poa pratensis* L.) compared to Kentucky bluegrass alone resulted in greater soil microbial diversity, different soil microbial communities, and fewer turfgrass pathogens.

Ecological theory suggests that increasing the number of species can enhance ecosystem functioning, and these benefits accrue over time. The insurance effect (Yachi & Loreau, 1999) details that additional species provide a safeguard to various biotic and abiotic stresses, since we know species are differentially adapted to particular stresses, and this has been demonstrated in field experiments (Minns et al., 2001; Tilman & Downing, 1994). Lehman & Tilman (2000) found that increases in plant species diversity stabilizes community productivity but results in decreased stability for individual species. Further research is needed to determine the relationship of turfgrass species diversity planted along roadsides and functioning holding true.

## Potential disadvantages of turfgrass mixtures

A disadvantage of turfgrass mixtures has historically been and continues to be a poor aesthetic. Juska & Hanson (1959) noted how mixtures of 'Merion' Kentucky bluegrass and tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort; syn. *Festuca arundinacea* Schreb.] had improved turf quality compared to a tall fescue monoculture for four years (1954-1957), but in the last year of the study (1958) the quality declined due to tall fescue clumping, a problem also observed by Brede & Duich (1984a). Maintaining a roadside similarly to a lawn can result in poor roadside vegetation, since it is likely being mowed too low (<7.6 cm), and not fertilized or maintained with irrigation; whereas if it was mowed at a higher height of cut (>7.6 cm) it could be mowed less frequently and have lower fertility needs, likely resulting in greater root biomass (Juska et al., 1955). Furthermore, roadside soils often contain poor soil fertility (Hopkinson et al., 2016; Mills et al., 2020) and this, in addition to poor management, will slow the growth of vegetation in these areas and reduce recovery. In general, based on the functional goals of roadside turfgrass, a nonuniform aesthetic should not be viewed as a limitation, except for roadside areas that are contained within or nearby residential or commercial lawns.

Public land managers and other turfgrass practitioners would benefit from additional research studying roadside turfgrass mixtures. To date, this work has been limited due to the high cost of conducting these trials and limited availability of turfgrass cultivars well-adapted to the multiple abiotic and biotic stresses found on a roadside (Friell & Watkins, 2020).

## General factors affecting the results of turfgrass mixture experiments

Testing turfgrass mixtures in the environments they will be utilized in is important for numerous reasons. Previous low-maintenance mixture studies, including some along roadsides, have regularly applied fertilizers (Bunderson, 2007; Dernoeden et al., 1998; Dudeck & Young, 1970; Dyrness, 1975; Miller et al., 2013), herbicides (Bunderson, 2007; Dernoeden et al., 1998; Miller et al., 2013), and sometimes supplied irrigation throughout the experiment (P. G. Johnson, 2003). These factors can alter species competitiveness disproportionately in a way different from the management of most roadsides. Species included for roadsides should be tolerant of higher salinity (Biesboer & Jacobson, 1994), poor and ill-timed management (White & Bailey, 1969), no supplemental irrigation, and little to no fertilizer inputs should be expected. Even though a roadside is a low-input environment, it does not imply that roadside vegetation should receive no routine maintenance (Hottenstein, 1969); rather, species should seldom be included for roadsides that are known to only thrive in higher input conditions.

When selecting turfgrasses to tolerate less inputs it is important to recognize that individual species and cultivars have a range of adaptations to differences in climate, soil, and management factors. Turfgrass species have different tolerances to fertility levels (Beard, 1973; Hunt & Dunn, 1993), timeliness of germination and robustness of establishment (Bunderson, 2007; Dunn et al., 2002), salt tolerance (Friell et al., 2013), heat tolerance (Breuillin-Sessoms & Watkins, 2020; Xu et al., 2018), ice tolerance (Guðleifsson, 2010; Watkins et al., 2018), drought tolerance or avoidance (Qian et al., 1997), and other abiotic and biotic stresses; therefore, an appropriate mixture needs to be designed to tolerate and thrive in any combination of these stresses.

The intensity, duration, and frequency of maintenance can have an impact on turfgrass mixture experiments (Watschke & Schmidt, 1992). For instance, some turfgrass species, such as bentgrass (*Agrostis* L. spp.), and weeds, such as smooth crabgrass [*Digitaria ischaemum* (Schreb)] and large crabgrass [*Digitaria sanguinalis* (L.) Scop.], are known to be better adapted to a lower height of cut (Davis, 1958; Dernoeden et al., 1998; Juska & Hanson, 1959). White & Smithberg (1972) found that the interval between mowing times is more significant than the mower type finding that smooth bromegrass

(*Bromis inermis* Leyss.) is more abundant on roadsides in Minnesota where mowing was less frequent. Hunt & Dunn (1993) found greater disease incidence at a lower mowing height in mixtures of cool-season grasses.

Soil and edaphic conditions can influence the results of turfgrass mixture experiments. At three Michigan roadsides sites, Martin & Kaufman (1970) found Kentucky bluegrass dominated a loamy clay site while strong creeping red fescue was the only significant grass remaining on the sandy site. The inclusion of tall fescue, redtop bentgrass (*Agrostis gigantea* Roth), creeping bentgrass (*Agrostis stolonifera* L.), orchardgrass (*Dactylis glomerata* L.), and smooth bromegrass provided no significant benefit at the three research sites tested. In Minnesota, White & Smithberg (1972) found when seeding mixtures of Kentucky bluegrass, redtop bentgrass, white clover (*Trifolium repens* L.), and perennial ryegrass (*Lolium perenne* L.) on roadsides that redtop bentgrass dominated sections with higher soil moisture, and Kentucky bluegrass and smooth bromegrass dominated the drier areas. Similar results with redtop bentgrass were reported by Foote et al. (1978). Duell & Schmit (1975) found turf-type Kentucky bluegrass and tall fescue performed poorly on high sand and low nutrient soils and there were general difficulties establishing grass at a site containing 96% sand. Despite slow establishment, hard fescue (*Festuca brevipila* Tracey) 'C-26' had consistently one of the best ratings at the end of the 5-year experiment; the authors' final recommendations for New Jersey roadsides included an even ratio of strong creeping red fescue, common-type Kentucky bluegrass, then either Chewings fescue [*Festuca rubra* L. ssp. *commutata* Gaudin; syn. *Festuca rubra* L. ssp. *fallax* (Thuill.) Nyman] or hard fescue. Foote et al. (1978) tested different mixtures at four roadside research sites and found it especially difficult to maintain turfgrass coverage on excessively sandy sites but found that sand dropseed [*Sporobolus cryptandrus* (Torr.) Gray], smooth bromegrass, Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski], and timothy grass (*Phleum pratense* L.) could be more suitable for these conditions. Henslin (1982) found sheep (*Festuca ovina* L.) and hard fescue dominated at an exceptionally dry site near Rice, MN. Henslin (1982) also presents some evidence showing superior varieties at one site had dissimilar performance at sites with different soil types but similar climates; a final recommendation included strong creeping red fescue, Canada bluegrass (*Poa compressa* L.), hard fescue, and sheep fescue for sandy well-drained roadside areas.

## Limitations of many turfgrass mixture experiments

There are many limitations and challenges to compare turfgrass mixture experiments on roadsides and other low-maintenance areas. Coverage is only evaluated for a few years or less (Engelhardt & Ratliff, 2019; Friell et al., 2012, 2015; Henensal et al., 1980), and in that period of time, the number, type, order, and duration of stresses may be lacking and therefore the analysis may result in poor recommendations. If an experiment collected longer-term data that would provide future advantages or disadvantages of some species (Damgaard & Weiner, 2017). For example, tall fescue (Friell et al., 2015) and perennial ryegrass (Friell et al., 2012) are susceptible to winter injury on roadsides in Minnesota, but if only evaluated for a year or less their results may be superior simply based on the severity of a single winter; a similar example was reported in a turfgrass shade experiment by Gardner & Taylor (2002). Turfgrass mixture experiments also usually test and recommend few species compared to non-turfgrass settings and there is a tendency to simplify turfgrass mixture (Boeker, 1970; Friell et al., 2015). In non-turfgrass settings, mixtures usually include more species and encourage the practice of complexifying mixtures (Barr et al., 2017).

It is also difficult to compare turfgrass mixture experiments because many design and mix species by weight. Consider the fact that a common turfgrass mixture, such as 90% tall fescue to 10% Kentucky bluegrass by weight, is nearly a 1:1 seed ratio. Weight is then generally more arbitrary. Furthermore, there are differences in seed lot purity, germination rate, and differences in seed size between species; cultivar within a species; and seed lots within a single cultivar (Christians et al., 1979). Mixing species by pure live seed or “field-viable seed” ratio has been recommended before (Brede & Duich, 1984a), since it contains more information than just weight. This allows for better comparison, but there are still differences in environmental conditions and maintenance procedures between experiments, and differences in seeding rate.

## Results of turfgrass species and mixture experiments

Testing of turfgrass species usually occurs in monoculture trials with the aim of identifying species and cultivar adaptation. After 6 years of evaluating coverage in West Virginia, Blaser (1964) found redtop bentgrass, strong creeping red fescue, and perennial ryegrass had poor coverage. In the upper Midwest, Diesburg et al. (1997) found tall fescue and sheep fescue generally performed the best. Buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] performed adequately in southern Illinois and in Ohio, and colonial bentgrass performed well at a few sites with lower fertility. One limitation of Diesburg et al. (1997) was that there were significant differences in soil quality between sites, which was confounded with the relative regional adaptation. In the north-central United States, Watkins et al. (2011) found that hard fescue then tall fescue both performed well in a two-year low-input turfgrass study. On the contrary, tall fescue's roadside performance in this region has been shown to be more limited (Friell et al., 2012, 2015; Watkins et al., 2019). This is likely due to prolonged ice encasement (Guðleifsson, 2010).

Testing of less-utilized species has occurred. In Manitoba, Mintenko et al. (2002) reported that blue grama [*Bouteloua gracilis* (Willd.) Lag.] and prairie junegrass [*Koeleria macrantha* (Ledeb.) Schult.], a cool-season species native to North America, showed consistent green color for the duration of a low-maintenance turfgrass experiment. On roadsides in Minnesota, prairie junegrass had poor establishment after one year (Friell et al., 2012). On roadsides in New England, prairie junegrass was initially not the best, but after two years maintained a steady coverage of 45%, whereas the rest of the species contained less than 25% coverage (Brown et al., 2010). Weeping alkaligrass [*Puccinellia distans* (Jacq.) Parl.] usually results in poor coverage in low-maintenance experiments (McKernan et al., 2001), but occasionally has good coverage along salted freeways when commonly tested turfgrass species are limited (Biesboer et al., 1998; Friell et al., 2012; Watkins et al., 2019).

Many turfgrass mixture experiments have occurred in combinations with two of either Kentucky bluegrass, tall fescue, or perennial ryegrass. Dunn et al. (2002) found in some instances, mixtures of Kentucky bluegrass and tall fescue performed better than a monoculture alone, due to greater disease resistance. Blaser (1964) found the inclusion of 'Kentucky 31' tall fescue and Kentucky bluegrass enhanced long-term coverage in a roadside mixture experiment. In a non-roadside mixture experiment in Minnesota, Miller et al. (2013) found that a blend of tall fescue performed better than a blend of fine fescues or a mixture of Kentucky bluegrass and tall fescue; a Kentucky bluegrass cultivar blend performed the poorest. Brede & Duich (1984a) found that the best performing perennial ryegrass and Kentucky bluegrass mixtures resulted in greater leaf area index, seedling density, ground coverage, and improved spring green-up compared to monocultures.

Mixtures of fine fescue species and Kentucky bluegrass have been previously recommended, likely due to similar competitiveness and therefore good complementarity. Juska & Hanson (1959) seeded 50 different turfgrass mixtures and found that for four years, 'Marion' Kentucky bluegrass monoculture was the best entry, but in the fifth year it significantly declined due to disease. When 'Merion' contained 25%, by weight strong creeping red fescue, then overall plot quality was stable during the disease pressure. Yuan et al. (2014) tested mixtures of Kentucky bluegrass, strong creeping red fescue, and alkaligrass and recommended a mixture of 32% Kentucky bluegrass to 68% strong creeping red fescue, by pure live seed weight. Kentucky bluegrass generally is more competitive under greater nitrogen fertility than fine fescue (*Festuca* L. spp.) species (Juska et al., 1955).

Examples of three or more species in turfgrass mixtures are more limited in the literature. In Missouri, Hunt & Dunn (1993) found mixtures consisting of tall fescue, perennial ryegrass, and Kentucky bluegrass had fewer weeds than a monoculture plot of tall fescue over the duration of a five year experiment; plots were maintained at a low height of cut (16 and 22 mm), and the abundance of tall fescue declined from 51 to 11% in a mixture with perennial ryegrass that was initially seeded at a rate of 8:1 by weight, respectively. In that same period, a mixture of tall fescue and Kentucky bluegrass remained stable. Larsen et al. (2004) found that a 3-way mixture of slender creeping red fescue [*Festuca rubra* L. ssp. *littoralis* (G. Mey.) Auquier], perennial ryegrass, and Kentucky bluegrass, which contained close to half of the viable seeds, that in less than a year, 3-30% of the stand contained Kentucky bluegrass. In Maryland, Dernoeden et al. (1998) found mixing tall fescue and strong creeping red fescue resulted in the best turfgrass quality in the fall for three years when mowed at 6.5 cm and no overall benefit was found in any seed mixture with different mowing treatments.

Multi-species mixtures have also been tested or are currently recommended on roadsides. Friell et al. (2015) found all species except tall fescue improved survival; additionally, they showed some evidence for poor complementarity between mixtures of alkaligrass and slender creeping red fescue and that may limit their use together in mixtures. The authors' final recommendation was limited to the tested constituent proportions, and they recommended the top three species consisting of hard fescue (40%), sheeps fescue (40%), and slender creeping red fescue (20%). The current recommended mixture 25-151 (conventional turfgrass) by the Minnesota Department of Transportation (MnDOT), has been recommended in a similar ratio since at least the mid to early 1990s, and it currently contains a mixture by weight of perennial ryegrass (17%), strong creeping red fescue (8%) and a triple blend of Kentucky bluegrass (totaling 75%) (MnDOT, 2014). Perennial ryegrass has been found to disappear rapidly on a roadside in cold climates and so its coverage is likely temporary (Friell et al., 2012; Watkins et al., 2019).

## Mixing cool and warm-season turfgrasses

The mixtures discussed above only included cool-season species. Mixing cool ($C_3$) and warm-season ($C_4$) turfgrasses may be useful in some turfgrass management situations as the benefits of both types could be attainted resulting in improved seasonal coverage, color, and greater stability to a variety of abiotic and biotic stresses. Roadsides in Minnesota are anecdotally known to contain disproportionately warmer and drier areas than surrounding vegetation, and so cold tolerant warm-season grasses have the potential to perform well in these areas in mid-summer. Usually, mixing warm and cool-season species together results in a patterned carpet look resulting in poor uniformity and difficulty in achieving long term persistence of both types (Davis, 1958; Dunn et al., 1994). Success has been found with mixtures of zoysiagrass (*Zoysia japonica* Steud.) and tall fescue (D. Li & Han, 2008; Xiang, 2018). However, as mentioned earlier, these aesthetic concerns should not apply for most roadside turfgrass settings.

Bunderson (2007) tested mixtures in Utah and found a mixture of the warm-season blue grama and the cool-season western wheatgrass [*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey] was successful since those species did not outcompete each other and both displayed color in their respective growing seasons. Additionally, a mixture of sheep fescue and buffalograss, a warm-season species, resulted in 90% sheep fescue in two growing years. Similar results have also been reported when seeding fine fescues and buffalograss in a mixture by Johnson (2003), who found buffalograss was dominated by fine fescues after a few growing years in Utah. In that study, the plots were irrigated at an evapotranspiration replacement of 50%, and the evenings are relatively cool in Utah for warm-season grasses, which may have favored the fine fescues over time. Severmutlu et al. (2005) in Nebraska found that when overseeding sheep fescue into buffalograss in the fall, the length of green color was extended by two months for the duration of the study, even though the final species composition was still dominated by sheep fescue (80% of total plot coverage). Similar results have been found on golf course fairways by Abeyo et al. (2009), but with more overall turfgrass shoot density when overseeding sheep fescue at 5 g $m^{-2}$ into a buffalograss stand. Several researchers have emphasized selecting compatible cool and warm-season species and cultivars when mixing warm and cool-season grasses (Abeyo et al., 2009; Menegon et al., 2017; Rimi & Macolino, 2014). Weeping alkaligrass has been tested and recommended with native warm-season turfgrasses in Minnesota (Stenlund & Jacobson, 1994), likely since its coverage can behave like an annual (Biesboer et al., 1998), supplying adequate coverage in spring before warm-season turfgrasses begin seasonal growth. Despite the mixed results of mixing cool and warm-season turfgrasses, there is still potential that a mixture of this type could persist for multiple years. The coverage type would likely oscillate in abundance with fluctuations in temperature, precipitation, and their interaction, and this may provide long-term benefits for roadside vegetation.

## Mixing turfgrass species with other functional groups

Mixing turfgrass species with non-turfgrass species can result in added benefits to a landscape. In a couple roadside experiments, turfgrass coverage was found to decline over time (Dudeck & Young, 1970; Dyrness, 1975) this was usually related to low fertility soils. To improve soil fertility, those researchers recommended regular fertilizer applications. The inclusion of legumes with turfgrasses may alleviate the need to regularly apply fertilizer along roadsides due to their association with nitrogen-fixing bacteria. Furthermore, the addition of nitrogen fertilizer has been found to reduce legume abundance (Dyrness, 1975; Nassiri & Elgersma, 2002).

White clover has commonly been included in turfgrass mixtures due to its adaptation to mowing. In fact, it was commonly included in lawn mixtures prior to the 1940s before the widespread use of synthetic fertilizers (Beard, 1973). The inclusion of white clover in mixtures with grasses have been found to improve the color and result in greater nitrogen concentration in plant tissue (Sincik & Acikgoz, 2007). When white clover was added to a roadside turfgrass mixture, the resultant sward was found to support an additional 61 pollinator species compared to a grass only roadside mixture (Burt et al., 2020). Evans et al. (1989) reported that coexisting mixtures of white clover and grasses resulted in more dry matter than types that were previously not coexisting together. White clover has also been found to establish in different soil types (Lane et al., 2019), one reason why it is widely adapted in North America. Historically, MnDOT has included white clover in a mixture equivalent to its current low maintenance turfgrass seed mixture (25-131), but it was not included in mixtures recommended in 2005 and thereafter (MnDOT, 2014). One concern with white clover is its susceptibility to prolonged drought stress (Norton & Morgan, 2020) and this may reduce coverage on dry roadsides. Overall, the inclusion of white clover with a mixture of turfgrass species can provide many benefits.

A mixture of perennial ryegrass and crownvetch [*Securigera varia* (L.) Lassen; syn: *Coronilla varia* L.] resulted in crownvetch dominating the mixture (Dudeck & Young, 1970), but in 2017 crownvetch was classified as a restricted noxious weed in Minnesota (Minnesota Department of Agriculture, 2021). Lane et al. (2019) tested the adaptation of other forb species with hard fescue and self-heal [*Prunella vulgaris* ssp. *lanceolata* (W. Bartram) Hulten], creeping thyme (*Thymus serpyllum* auct. non L.), and ground plum (*Astragalus crassicarpus* Nutt.) and some species showed good potential in a non-roadside situation. Birdsfoot trefoil (*Lotus corniculatus* L.) was tested in roadside turfgrass mixtures (Foote et al., 1978; Johnson et al., 1971) and has even been recommended for this use (Hottenstein, 1969), but has been removed from recommended seed mixtures in Minnesota, likely due to its weedy characteristics. Carpet vervain [*Verbena bracteata* Lag. & Rodr.] has been observed growing along roadsides that are frequently mowed in MN (D. Christensen, personal observation) and a perennial type could have potential for good mixability with turfgrass species. One limitation reducing the ability of mixing forbs along roadsides is they tend to have poorer germination in conditions of high salinity, especially in the first 3 m from the edge of the road (Biesboer & Jacobson, 1994).

An area of mixture development that is relatively unexplored is mixing turfgrass species with sedges and rushes. Sedges and rushes are often difficult to distinguish due to their grass-like appearance (Smith, 2018). There is good mixture potential of these species with turfgrasses. For instance, there are sedge and rush species that are short stature and rhizomatous (Smith, 2018), which would make them good candidates with turfgrass species. Path rush (*Juncus tenuis* Willd.), for example, is known to have excellent wear tolerance and has been observed growing with weeping alkaligrass along a roadside in International Falls, Minnesota (D. Christensen, personal observation). One limitation of path rush is it has been identified as potentially weedy (Engelhardt, 2016), and this could limit its use. Nonetheless, both sedge and rush species deserve more attention and testing for use in turfgrass mixtures for roadsides and other landscapes.

## Designing turfgrass mixtures

The design of a turfgrass mixture and most seed mixtures can often be viewed as an art. There may be multiple appropriate ways to mix the type, number, ratio, and rate of species, but there usually are many incorrect ways. Proper design of a turfgrass mixture considers many aspects. An appropriately designed turfgrass mixture would be tolerant to or avoidant of many abiotic and biotic stresses and poor management practices that often occur along roadsides. An effective mixture would also be able to withstand dynamic and different future stresses; it is important to consider the climate, soil conditions, and disturbance or management factors, and the timing of seeding relative to the respective growing year. After these considerations, then the type of species can be selected, the number of species, number of cultivars for each species, appropriate ratios of these species, and the seeding rate.

**Identifying the heterogeneity of a seeding area**

A large area or region contains more variation in climate, soil, disturbance, and management factors. Therefore, an important consideration is identifying if a mixture should be designed to an individual site level (Kirmer et al., 2012; MacDonagh & Hallyn, 2010) or to a broader region. Consider an example of recommending a mixture when there are site differences for mowing height and frequency: one city could maintain its roadside vegetation at a low cutting height of 5.1 cm and a nearby municipality at 8.9 cm, both mowing once per week. Perhaps the next two towns on the same highway mow their roadsides at the same heights, but both have frequencies of every two weeks. These four cities could all be next to one another experiencing a relatively similar climate, but the management factors alone will disproportionately favor the competitiveness of different weedy and turfgrass species (Davis, 1958). Therefore, if a turfgrass mixture is to be recommended covering a large region with more heterogeneity in climate, soil factors, and differences in disturbance, then it would be obvious to modify the mixture to include species to accommodate a range of factors. If the design of a roadside seed mixture focused too heavily on a single species and that species suffers, whole failure could ensue from that mixture. Turfgrass mixtures for roadsides have been recommended for different moisture regimes (Boeker, 1970; MnDOT, 2014). Testing has also occurred for differences in elevation (Hopkinson et al., 2018) and region (Engelhardt & Ratliff, 2019) which both relate to the climate and soil characteristics. Species and cultivar survival has also been tested with different soil amendments (Brown & Gorres, 2011). The heterogeneity within a region or site may be large or small depending on the conditions and asking these questions is a beneficial beginning when designing a turfgrass mixture.

## Seeding timing

The timing of seeding will modify how a mixture is designed. We know there are optimal periods to seed cool-season turfgrasses (Braun et al., 2021; Minnesota Department of Highways, 1962; Watkins & Trappe, 2017). Seeding timing is important because weed pressure cycles throughout the growing year. Natural weed pressure is lower in the fall, since warm-season annuals have largely concluded their life cycle, but soil temperatures are still relatively high, and this allows for sufficient turfgrass establishment with less weed pressure. When seeding cool-season mixtures in non-recommend seeding timing, previous recommendations have included perennial ryegrass (Henensal et al., 1980) since it establishes faster acting as a temporary cover crop reducing the abundance of weeds. Perennial ryegrass will, however, modify the overall competitiveness of the mixture (Engel & Trout, 1980) which could result in over emphasizing short-term coverage of a mixture. The timing of seeding mixtures containing both cool and warm-season turfgrass species likely requires more research on the type of species, cultivars, ratios, and fall management before winter. The optimum growth rate for most cool-season species is between 16-24°C and 27-35°C for warm-season species (Woods, 2013). Differences in optimum growth will lead to non-uniform competition based on the season. In general, warm-season grasses are more susceptible to winter kill if seeded in the fall, without sufficient establishment prior to winter (Fry et al., 1993; Li et al., 2016; Patton et al., 2004). Other options could consist of applying a preemergent herbicide before seeding, sodding, or seeding a warm-season turfgrass and allowing it to establish and grow for one growing year, then seeding into the thatch from winter kill in the following spring (White & Smithberg, 1972). Seeding timing needs to be considered to balance the short and long-term coverage during establishment.

### Selecting the type of species

The type of species being selected for an area is an important factor after identifying characteristics of the planting area and timing of seeding. Watschke & Schmidt (1992) reported that when beginning to develop a turfgrass landscape it is important to begin with the most adapted species for the climate, environmental stresses, intended function, and maintenance level. Doing this will also result in one of the most important cultural control of weeds (Busey, 2003). In general, species in a mixture should be ones that are relatively compatible with one another, implying that they do not necessarily outcompete each other rapidly (Bunderson, 2007). In a mixture, all species should have the opportunity to establish, unless if a species is included solely for insurance; implying that it will establish only if the others fail. In Nebraska, for example it has been shown that smooth bromegrass, Kentucky bluegrass, and tall fescue compete, invade, and threaten the establishment and persistence of native warm-season grasses (Soper et al., 2019a), and so the general compatibility of these species in a mixture would be relatively low.

Ecological theory would suggest that greater diversity in the type of species, noted as asynchrony (Yachi & Loreau, 1999), effectively defends against more stresses. Greater asynchrony, in the context of roadside mixtures, may mean that a mixture should include greater diversity than just fine fescue species (Friell et al., 2015) or other simple mixtures (Engelhardt & Ratliff, 2019). Mixing other adapted cool season species from other genera, warm-season grasses, and species from other functional groups may provide added benefit over space and time than a group of species behaving similarly. Engelhardt & Ratliff (2019) found differences in regional coverage of species and recommended two warm-season grasses at the central location, since those species established well and resisted invasion of summer weeds, but at the eastern location there was not any good performing seed mixtures, which the authors attributed to excessive weed invasion of crabgrass and foxtail (*Setaria* spp.), along with low plant available soil moisture.

## Selecting an appropriate number of species

More species that are included in a mixture usually results in benefits in both non-roadside (Dunn et al., 2002; Johnson, 2003; McKernan et al., 2001; Tyser et al., 1998; Xiang et al., 2019), and roadside environments. Biesboer et al. (1998) found that a fall seeded mixture of warm-season natives, cool-season natives, and cool-season introduced species, when seeded along a roadside in Cambridge, Minnesota had the better cover after two years compared to either a warm-season or a cool-season nonnative mixture alone. Henslin (1982), also based on research in Minnesota, found that mixtures usually performed better if the top monoculture was included in a seed treatment illustrating one benefit of the insurance effect (Yachi & Loreau, 1999). Additionally, the testing of some wheatgrass species (*Triticeae*) shows they may have relatively poor coverage by themselves, but when seeded with Kentucky bluegrass, can add to improving the coverage and density of turfgrass mixtures (Robins & Bushman, 2020).

## Cultivar selection and number

The selection, type, and number of cultivars in a mixture is important, since adapted cultivars can also result in few weed problems (Busey, 2003). Some roadside experiments have attempted to identify cultivars that are the most suitable for roadsides and differences have been found based on the age of the cultivar of some species (Friell & Watkins, 2020). There have been experiments attempting to identify the most adapted roadside cultivars in controlled environments (Biesboer & Jacobson, 1994; Breuillin-Sessoms & Watkins, 2020; Friell et al., 2013; Watkins et al., 2018) and field experiments (Brown & Gorres, 2011; Friell et al., 2012; Watkins et al., 2019). Results of these studies show differences within individual species; however, Brown & Gorres (2011) found that cultivar differences may not necessarily matter to the long-term performance at a roadside site if it contains poor soil conditions. There may be differences in mixability of cultivars within different species. This is rarely tested, but variation likely exists, since it has been shown in wheat (Knott & Mundt, 1990; Lopez & Mundt, 2000), soybean (Gizlice et al., 1989), and has been discussed in a review paper (Barot et al., 2017), and so the types and characteristics of turfgrass cultivars that allow for greater mixability should be explored.

The number of cultivars should also be considered, since benefits could be gained by blending cultivars. This need is identified from the fact that certain cultivars such as ‘Merion’ in one study can have superior quality for several years, but then suffer from unexpected disease (Juska & Hanson, 1959). Several experiments have tested blends of turfgrass species in monoculture or in mixtures but do not compare individual cultivars against one another (Dunn et al., 2002; Hunt & Dunn, 1993; Miller et al., 2013). One Kentucky bluegrass variety, ‘Award’ was superior in a monoculture to almost all blends when evaluating it for a few years, additionally one poor cultivar when included in a blend can reduce the overall quality (Brede, 2007). Similar results were observed by Brede (2004) who showed that blending can reduce turfgrass quality, although these results may be limited since testing occurred at a single location. Oral & Açykgöz (2001) showed that blends of most cultivars usually result in the average of each individual cultivars quality. Blends of Kentucky bluegrass have been shown to not necessarily result in increased sod strength, but some three-way blends significantly improved sod strength (Hall III et al., 1985). One currently recommended roadside turfgrass mixture by MnDOT contains a triple blend of Kentucky bluegrass (MnDOT, 2014) and a mixture recommended for Maryland contains two cultivars of tall fescue (Engelhardt & Ratliff, 2019). It is unknown how these blends would perform over time and space on roadsides if they were reduced to a single cultivar, but they would likely perform worse especially if the individual cultivar was not tolerant to conditions common to roadsides. Hanson et al. (1952) found that mixing three cultivars of Kentucky bluegrass resulted in more grain yield than a single cultivar. In general, the greatest benefits in the design of turfgrass mixtures would likely come from greater asynchrony first (Yachi & Loreau, 1999) and so species diversity should be prioritized before cultivar diversity when designing turfgrass mixtures.

### Selecting species ratios

Even if the appropriate type of species, number, and most adapted cultivars were selected, the designated ratios of different species in a mixture is highly important to success. A poorly designed mixture could result in excessive dominance by one species. An appropriate ratio of species in a mixture is one that allows for adequate short-term coverage whilst not overwhelming the potential of species that establish slower.

Friell et al. (2015) suggested a mixture by seed of 20% slender creeping red fescue, and 40% of both hard and sheep fescue would be a sufficient mixture for a roadside in central Minnesota. Engelhardt & Ratliff (2019) found a 2:8 mixture of tall fescue to fine fescue by weight was suitable for a roadside in western Maryland. Hottenstein (1969) recommended mixtures for different regions in Minnesota. The frequent mowing mixture recommended for northwest Minnesota included perennial ryegrass (3.36 kg $ha^{-1}$), Kentucky bluegrass (2.24-6.73), blue grama (2.24), and buffalograss (5.60-28.02). The eastern Minnesota recommendation contained one mixture for rural and another for urban areas. The rural mixture consisted of tall fescue (28.02), Kentucky bluegrass (8.97), perennial ryegrass (8.97), birdsfoot trefoil (3.36), and white clover (3.36). The urban mixture contained Kentucky bluegrass (44.83), perennial ryegrass (8.97), strong creeping red fescue (20.18), and redtop bentgrass (8.97). This report did not specify how the species and ratios were selected and a definition and reason for the distinction between urban and rural areas. Currently, it is easier to show examples of poorly designed mixture ratios than to identify optimum ratios.

One of the most common mistakes in the design of cool-season turfgrass mixtures is including species that establish too quickly and robustly in high proportions, thereby dominating the stand and not allowing for other well-adapted species to thrive. Perennial ryegrass included at 10% or greater by weight and even less depending on which species it was seeded with, greatly interferes with other species (Dunn et al., 2002; Henensal et al., 1980). Brede & Duich (1984a) found that an optimum mixture of Kentucky bluegrass to perennial ryegrass ranged from 70-95% field viable seeds of Kentucky bluegrass. This allowed for good establishment and low perennial ryegrass clumping. However, perennial ryegrass' inhibition on other species in a mixture cannot be overstated, since it can result in reduced plant sizes of Kentucky bluegrass, strong creeping red fescue, and colonial bentgrass when seeded together (Engel & Trout, 1980). Great care must take place to limit its seed ratio in mixtures to not reduce the competitive ability of these longer-term grasses. Limiting the use of aggressive seedlings has also been recommended before for roadsides (Blaser, 1964).

Other species included in a mixture at even a low rate can also have a significant effect over time. Foote et al. (1978) tested roadside mixtures in the metropolitan area of Minnesota and found that when reed canary grass (*Phalaris arundinacea* L.) was included at a pure live seed rate of 3.6%, its overall coverage was found to increase over time. In a roadside experiment in Texas, Simmons et al. (2011) found curly mesquite [*Hilaria belangeri* (Steud.) Nash var. *belangeri*] seeded at a rate of 0.1% by weight (in a mixture with other warm-season grasses and nine vegetative plugs) had a coverage close to 30% after 2 years, although vegetative cultivars can perform better than seeded cultivars (Croce et al., 2001), which could have impacted those results. In a turfgrass mixture experiment, Juska & Hanson (1959) found a mixture containing only 7% strong creeping red fescue and 26% common Kentucky bluegrass consisted of 47.5 and 42.5% coverage in five years, respectively; if a mixture contained 5% colonial bentgrass by weight, it resulted in 100% colonial bentgrass at the conclusion of the study. In addition to the low mowing height in that study, the species were mixed by weight, and it is known that bentgrass seeds are much smaller than many cool-season turfgrasses (Beard, 1973). If these mixtures were designed originally by seed ratio, then colonial bentgrass would have contained far fewer seeds, potentially reducing its future abundance. Juska et al. (1955) found when redtop bentgrass was lower than 10% in a mixture then it did not outcompete ‘Merion’ Kentucky bluegrass. Hsiang et al. (1997) found relative to the original seed mixtures by weight, perennial ryegrass and Kentucky bluegrass were more abundant, fine fescue was similar, and tall fescue was less abundant. Kentucky bluegrass and fine fescue moved towards an equilibrium point over time from 35 to 42% Kentucky bluegrass and 24 to 25% fine fescue. White clover is also included in many states’ department of transportation roadside turfgrass mixtures from 1-11% (Busby, 2014). The seeding rate of white clover is typically a small value in a mixture, since it contains very small seeds and can be highly competitive from its stolons. The evidence is strong that turfgrass seed mixtures should be designed using an appropriate pure live seed ratio rather than just by weight; this would allow a mixture to be more precisely designed, tested, and compared across different experiments.

### Seeding rate

Another aspect to designing a turfgrass mixture is the seeding rate. Patton et al. (2004) found greater coverage with higher seeding rates in two warm-season grasses at first, but after 42 and 70 days of seeding bemudagrass [*Cynodon dactylon* var. *dactylon* (L.) Pers.] and zoysiagrass the coverage was the same for all seeding rates, although the authors anecdotally observed greater density of higher seeding rates and lower biomass of individual tillers, similar to Lush (1990). Christians et al. (1979) found that the same weight of seeding smaller-seeded cultivars of Kentucky bluegrass compared to larger-seeded cultivars resulted in no difference in coverage after 6 growing months. Stenlund & Jacobson (1994) found no differences in coverage between two identical seed mixture ratios, except that one was seeded at two times the seeding rate for roadsides. L. Li et al. (2016) found that buffalograss seeding rate also had no impact on coverage.

It is difficult to identify an appropriate interaction of the seeding rate with the species ratio in a mixture. For example, if perennial ryegrass is recommended for a mixture at a low seed ratio, but the total seeding rate is overly high, then more perennial ryegrass seedlings will be seeded per unit area. This is particularly concerning, since that could reduce the efficacy of a turfgrass mixture (Dunn et al., 2002; Henensal et al., 1980).

Sloped sites are another situation where seeding at higher rates have traditionally been recommended. A higher seeding rate achieve greater tiller density more rapidly. Booze-Daniels et al. (2000) also recommends the importance of including a sufficient portion of companion or nurse crops on slopes in addition to recommended perennial species. This would allow for initial establishment to reduce erosion and then to later allow for the establishment of perennial species. This is similar to Henensal et al. (1980) recommendation for perennial ryegrass in situations where there is high erosive potential.

Overall, the seeding density between different mixtures of turfgrass species needs more testing to identify appropriate seeding rates for different types of mixtures.

## Conclusion

This review covered the purpose, function, consequences, and design of seeding turfgrass mixtures with an emphasis on roadsides. An appropriately designed mixture is one that can provide adequate short and long-term erosion control, result in an aesthetically uniform and pleasing landscape, be cost efficient, and to serve drivers in safer movement and transportation. The testing and evaluation of turfgrass mixtures is not likely to be exhausted, since the combinations of species, ratios, seeding rate, place, management, climate, and other environmental factors are very numerous. More research on turfgrass mixtures evaluated over longer periods of time will allow for more information to be gathered and then disseminated. This will result in new and improved mixtures for roadsides, parks, and other low-maintenance areas, benefiting not only man, but the environment and place in which he occupies.

# Chapter 2

# Minnesota Roadside Seed Banks and their Impact on a Seeded Turfgrass Mixture Study

## Summary

Establishment and maintenance of vegetation along roadsides in Minnesota are challenging. The abundance and diversity of species in seed banks contributes to this challenge, thus we sought to identify and characterize the seed bank vegetation along roadsides and determine their impact on weed coverage while testing different seeded turfgrass mixtures. We conducted a seed bank analysis using the soil emergent method in the greenhouse from soil collected prior to turfgrass establishment along roadsides at 14 sites throughout Minnesota adjacent to 2-4 lane roads. After soil collection, all sites were seeded for a turfgrass mixture experiment. Seven sites were seeded per year in 2018 and 2019 with 40 total seed treatments ranging from monocultures to a six-way mixture. We found differences at sites in both seed bank seedling density (23-209 seedlings $L^{-1}$) and observed species density (8-16 seedlings $L^{-1}$). We also found a much greater proportion of non-native to native emerged seedlings (0.74 to 0.17), but that disparity was lower for observed species richness (0.48 to 0.41). Observed seed bank species density was found to increase the odds of field weed coverage by 1.11 ($P=0.02$), but the significant interaction effect with time, defined as the order of vegetation sampling, indicated that the odds of that effect decreased by 0.97 times for each sampling instance ($P=0.03$). The effect of turfgrass and bare soil coverage were much more significant on weed coverage ($P<0.001$) than the seed bank covariate, indicating observed seed bank species richness has a relatively low impact on weed coverage. The results of our experiment suggest that roadside seed banks are unique at different sites in Minnesota, but they are not a major factor impacting the amount of weed coverage in late summer-seeded cool-season turfgrass stands.

## Introduction

Seeding and maintaining roadsides with turfgrass species reduces visibility impairments, soil erosion, and improves the aesthetics of these areas. Turfgrass establishment could be affected by undesirable species that emerge from the soil seed bank during or after the establishment period. The seed bank is defined as the total number and type of viable seeds contained in the soil, but usually there are only a subset of the viable seeds that germinate. Identifying characteristics of a roadside seed bank that may inhibit, limit, or reduce seeded turfgrass coverage would be valuable for practitioners. Important characteristics of a seed bank could be related to the diversity, density, and their interaction.

Seed banks have limited the coverage of seeded turfgrass on roadsides, largely in the case of high abundance of warm-season annuals (Biesboer et al., 1998; Engelhardt & Ratliff, 2019). Annual species are generally more common in the seed bank than in the aboveground vegetation (Bernards & Morris, 2017; D'Angela et al., 1988; Kalamees et al., 2012) since a primary mechanism for annual plant survival is through long-term seed persistence (Thompson, 1993). Seed banks are also known to contain greater abundance of smaller seeded species when sampled at a variety of locations and habitats (Kalamees et al., 2012; Thompson & Grime, 1979). This is the case since larger seeded species may be more susceptible to predation (Thompson & Grime, 1979).

Roadsides are impacted by a number of abiotic disturbances including ice encasement, snowplow damage, application of salt to roadways (Biesboer & Jacobson, 1994), and poor mowing maintenance (White & Smithberg, 1972); these factors collectively reduce the abundance of roadside vegetation over time (Friell et al., 2015). This leads to roadside seed banks containing more non-native and ruderal-like species in the aboveground vegetation (Bernards & Morris, 2017; Grime, 1973; Pauchard & Alaback, 2004). Abundance of non-native species along roadsides can function as a seed source to promote their unwanted spread to adjacent habitats (Fowler et al., 2008; Mortensen et al., 2009). In an experimental botanical garden, the intensity of abiotic disturbance was found to increase the similarity between the seed bank and the aboveground vegetation over time (Dölle & Schmidt, 2009). Considering that roadsides experience significant and regular disturbance, it is likely that a roadside seed bank and aboveground vegetation would be the most similar relative to all other ecosystems (Hopfensperger, 2007). Milakovic & Karrer (2016) found that the timing of roadside mowing, the frequency, and the height of cut can all significantly influence the emergence, establishment, and effective dispersal of certain weeds, because seeds can be dispersed by cars: the volume and direction of vehicle traffic significantly influences weedy dispersal along roadsides (Ansong & Pickering, 2013; Lemke et al., 2019; Vakhlamova et al., 2016). Vakhlamova et al. (2016) also found that the proximity from the city was found to influence seed bank composition rather than the road type, and that more non-native species were found along national roads in both the aboveground vegetation and the seed bank than at local roads. Those findings were attributed to habitat conditions and the origin of vehicles. An additional feature common to roadsides in cold climates is disturbance from snow plowing, which has been shown to result in more perennial forb cover (Pakeman & Small, 2005).

The abundance and type of vegetation that grows on roadsides is often different than in non-roadside areas. A common theme limiting vegetation along some roadsides is poor soil quality (Hopkinson et al., 2016). Plant trait responses to soil quality adjacent to roadsides has been shown to favor more nutrients, higher temperatures, and increased alkalinity (Williams et al., 2015). The distance from the road also results in different soil characteristics: as the distance increases, soil metals, sodium concentration, and bulk density decrease, while organic matter content, and water infiltration increase (Bhat et al., 2020; Mills et al., 2020)**.** In a survey of vegetation along Minnesota freeways subject to salting, Biesboer et al. (1998) found low coverage especially in the first meter consisting of a number of weedy annuals, and significantly greater total and perennial coverage three meters away from the road; additionally, more coverage was found on local roads with less traffic and disturbances. A New England multi-site survey found that 55% of identified species were non-native and as the distance from the road increased, the amount of perennial coverage was found to increase (Brown & Sawyer, 2012). We can therefore expect that the vegetation growing adjacent to a roadside is highly influenced by a combination of factors including soil characteristics, type and frequency of disturbance, and environmental effects.

Roadside seed banks, and their effect on seeded vegetation is poorly understood. Understanding the identity and abundance of a soil seed bank may inform the design of seeded mixtures for roadsides, seeding timing, and short or long-term maintenance. Therefore, the objectives of this study were to (i) characterize the seed bank from 14 roadside research sites in Minnesota, and to (ii) understand if characteristics of these seed banks affect the weed coverage of seeded turfgrass mixtures.

## Materials and methods

### Selection and establishment of field sites

Research site selection, turfgrass seed mixture design, plot preparation, seeding, maintenance, and more detailed field data collection, including percent weed and seeded turfgrass cover, are contained within Chapter 3. Field coverage was evaluated at each research site twice per year using the quadrat-grid intersection method. Sampling occurred once in the spring (April-June) and once in the fall (September-November).

### Seed bank sampling and testing

Seed bank soil was collected and composited from each of 3 blocks (repetitions) at 14 research sites after tilling the roadside area for turfgrass seeding. At sites seeded in fall of 2018, soil was collected by hand skimming the surface at regular distances to a depth of 2.5-10.2 cm every 5-10 m. Soil from sites seeded in fall 2019 was collected within three weeks after seeding using a cup cutter (Thompson, 1993); a total of 5 cores were sampled per block to a depth of 5.4 cm containing a total volume of 477 $cm^3$ per core. For improved sampling procedures the reader is directed to: Bigwood & Inouye (1988); Thompson et al. (1997); and Warr et al. (1993).

After collecting the soil from the field, 2018 and 2019 seed bank soil was vernalized to improve germination (Gross, 1990) in a refrigerator for 146 and 59 days, respectively. The average daily temperature in the refrigerator was approximately -1 °C for 60 days and approximately -7 °C for the remaining duration for fall 2018 soil and -2.7 °C for fall 2019 soil. After vernalizing, samples were allowed to air dry approximately 4 weeks at room temperature and then the soil was sieved through a 4-mm screen. The average weight of soil over blocks at each research site with standard deviation was 1073 ± 272 g and 2465 ± 402 g at fall 2018 and 2019 sites, respectively. Three subsamples each weighing 200 g were then sampled within each block for a total of 600 g per block and a total of 1800 g for each research site. That totaled 9 experimental units for each of 7 research sites for a total of 63 experimental units tested in the greenhouse using the soil emergent method (Thompson & Grime, 1979). Greenhouse containers were chosen with a surface area of 100 $cm^2$ for the 2018 and 95 $cm^2$ for the 2019 seed bank testing. This closely approximated a 2 cm thick layer of sieved field soil (Thompson & Grime, 1979) placed over unsterilized sand, but since testing occurred by weight some samples were slightly more or less than 2 cm. Samples were fertilized one time at a rate of 27.2 N-21.4 P-49.7 K kg $ha^{-1}$ using a 10-18-22 fertilizer (EC Grow Prolinks, EC Grow, Eau Claire, WI) mimicking field fertilization rate. Greenhouse containers were arranged in a completely randomized design and were rotated regularly to account for microclimate differences in the greenhouse. Unseeded control pots were also included to identify if unsterilized sand contained any weed seeds.

For the duration of the experiment the greenhouse had an average daily high of 27.7 °C and low of 18.7 °C for fall 2018 and 27.0 °C and 19.7 °C for fall 2019 sites. A total of 16 hrs of light was supplied from natural and supplemental sources each day. Seedling emergence was evaluated weekly for a 12-week period (Vakhlamova et al., 2016), and only vascular plants were identified. We found significant moss buildup over time that affected samples from some research sites and may have limited some seedling emergence. If seedlings could not be identified, they were transplanted and allowed to flower. Plant species nomenclature, characteristics, and identification was primarily based on Chadde (2019) with the exception of *Juncus* spp. identification, which was based on Känzig-Schoch et al. (2007) and Smith (2018). One seedling, *Potentilla argentea*, emerged from the control tray from the fall 2018 test and so that species was not counted if it emerged within that testing year. Small dropseed (*Sporobolus neglectus* Nash) was not distinguished from poverty dropseed [*Sporobolus vaginiflorus* (Torr.) Wood]. Additionally, 5 research sites from the 7 sites seeded in fall 2019 had one non-seeded field control plot to compare aboveground vegetation with seed bank results, if desired.

### Soil sampling and analysis

Soil samples were collected from research sites in August or September. Soil was collected before tillage by sampling 15-20 cores in each block in a zig-zag pattern at each site to a depth of 10-15 cm. All soil samples were composited by block within each site and analyzed for pH, soil texture, organic matter, extractable phosphorus, K, saturated paste extract electrical conductivity, Fe, and Mn. Additionally, Zn and Cu were included in soil tests due to their relationship of emergence and abundance of common weedy roadside vegetation (Bae et al., 2015). Total bulk density was determined within all three blocks at each site by utilizing the water volume determinant excavation method (Page-Dumroese et al., 1999). Soil analyses by block are shown in Table 2.1. Additional soil sampling details and results are included in Chapter 3.

### Statistics

Seedling counts for each subsample within each research site and block are shown in Table 2.2. The Chao species richness was calculated for each site to estimate the total number of species that each site likely contained in the seed bank while accounting for sampling limitations (Chao, 1984). The probability of an interspecific encounter (PIE) was calculated to have a standardized evenness measurement (Gotelli, 2008; Hurlbert, 1971). Seedling density was calculated by controlling for the total soil bulk density and expressing the value as seedlings $L^{-1}$ to account for variation in seed bank sampling depths between testing years (Stark et al. 2003). Expressing seedling counts by volume avoids the dependence on sampling depth for the seedlings per area reporting (Thompson et al., 1997), yet it may not be as intuitive as seedlings per area commonly reported. Species density was also calculated (James & Wamer, 1982).

All analyses were performed using the open-source software R (R Core Team, 2021). A non-metric multidimensional scaling (NMDS) was applied using the *metaMDS* function to spatially visualize the rank-order differences in species abundance and research sites. The *metaMDS* function and all subsequent functions for multivariate analysis were contained within the vegan package (Oksanen et al., 2020) and used a Bray-Curtis dissimilarity index. The *metaMDS* function was set with two dimensions with 100 attempts to find a solution, and the values were autotransformed to improve the results with a square root and then a Wisconsin double standardization transformation applied. The dimensions were set so the stress value of the NMDS did not exceed 0.2. The ordination analysis only included plants identified to at least the genus level. A PERMANOVA analysis was performed using the *adonis2* function to identify if there were differences in seed bank species composition by region. The regions tested in the analysis were ecoregion level 2 and 3 (Omernik, 1987; Omernik & Griffith, 2014), one of the 8 different MnDOT regions, and a custom separation from NNE sites to SSW sites, which included East Grand Forks classified by NNE sites and Fergus Falls and St. Cloud included within the SSW sites (Fig. 2.1). The homogeneity of variance assumption between regions was assessed by using the *vegdist* function on the matrix of the species composition data by site, and then applying the *betadisper* function.

A generalized linear model (GLM) with field weed coverage (%) as the response variable was conducted using the *glm* function with family set to binomial. All main effect explanatory variables included in the full model consisted of seeding year (2018 or 2019), time; defined as the order of vegetation sampling (1-5), research site, and the primary covariates were seed bank seedling density (count $L^{-1}$), Chao species density (count $L^{-1}$), observed species density (count $L^{-1}$), field turfgrass cover (%), and field bare soil cover (%). Interaction effects included time by observed seed bank species density, seeding year by time, time by seedling density, and time by Chao species density. To identify the final model, both a forward and backward stepwise model selection procedure was performed using the *stepAIC* function contained in the MASS package (Venables & Ripley, 2002). McFadden pseudo-$R^2$ values were calculated (McFadden, 1973) and used in addition to AIC score to compare model selection. Results are discussed in terms of odds, instead of log-odds, which is found from exponentiating the coefficient from the GLM table.

## Results

### Seed bank characteristics

The total number of emerged seedlings from the soil seed bank sampled from the field and emerged in the greenhouse ranged by site from 37 (East Grand Forks) to 318 (Marshall), with a total of 74 species identified across all sites. Observed species richness ranged from 10 at Edina to 21 at Roseville. Chao estimated species richness was found to double or more than triple the observed species richness at some research sites (Table 2.3), which suggests significant sampling limitations at some sites. The probability of an interspecific encounter was the lowest at Marshall and Roseville was next highest, indicating more dominance of seedlings by a few species (Table 2.3) at those two sites. Seedling density (seedlings $L^{-1}$) ranged from 23 (East Grand Forks) to 209 (Marshall), respectively, and this value was calculated by controlling for bulk density, since seed bank testing occurred by weight, resulting in sites with lower bulk density containing greater volume of soil tested. Observed species density was highest for Bemidji (16 species $L^{-1}$) and lowest for Edina (8 species $L^{-1}$). The Chao estimated species richness was highest for Grand Rapids (32 species $L^{-1}$) and lowest for East Grand Forks (9 species $L^{-1}$) (Table 2.4).

The average proportion of seedlings that emerged was much less for native seedlings (0.17) compared to non-native (0.74), yet the proportion was more similar for emerged species that were native (0.41) compared to non-native species (0.48) (Table 2.5). Similarly, the proportion of annual seedlings dominated most sites, but in terms of species number, the disparity between annuals and perennials was much smaller (Fig. 2.2). The top five species that appeared at the most sites were poverty dropseed found at 10 sites, large crabgrass found at 9 sites, and then broadleaf plantain (*Plantago major* L.), smooth crabgrass, and Kentucky bluegrass each found at 8 sites. The top five most abundant species by proportion were large crabgrass (0.35), black medic (*Medicago lupulina* L.) (0.09), smooth crabgrass (0.08), broadleaf plantain (0.04), and common purslane (*Portulaca oleracea* L.) (0.04). The PERMANOVA analysis found a significant difference only between the NNE-SSW separation ($F_{1,12}$=2.18, $R^2$=0.15, P=0.003). The NMDS results are shown for species separation in (Fig. 2.3A) and research site separation in (Fig 2.3B).

### Identifying the potential impact of the seed bank on field plot weed coverage over time

The results of the GLM indicated significant main and interaction effects on weed cover measured in the field plots. The planting year by time interaction suggests that weed coverage in the field was more similar at sites seeded in 2019 as time progressed compared to sites seeded in 2018 (odds increase by 1.25 times for each increment of time). The seeding year coefficient main effect odds estimate decreases 0.49 times illustrating that there is less weed coverage at sites seeded in 2019. While holding the other parameters constant, with each additional increment of time within a year, the odds of weed coverage on field plots increased 1.54 times (Table 2.6). With each additional increment of turfgrass or bare soil coverage, the odds of weed coverage decreased by 0.002 and 0.001 times, respectively (Table 2.6). Observed seed bank species density was found to increase the odds of weed coverage on field plots by 1.11, but the significant interaction effect indicated that the effect decreased over time (odds decrease by 0.97 times for each additional increment of time) (Table 2.6). Chao estimated species density and seedling density were not included in the final model based on the stepwise procedure. Most of the deviance in weed cover was explained by turfgrass and bare soil coverage, then time, and very little by observed seed bank species density (Table 2.7).

## Discussion

We found differences in many seed bank characteristics, despite all sites being located along a relatively well-drained roadside in Minnesota (Table 2.3-4; Figure 2.2-3). Most surprising was that weed coverage in plots was not impacted by seed bank seedling density, despite measuring 9 times higher seedling density at Marshall compared to East Grand Forks (Table 2.4). When examining the covariates of the GLM graphically, the average weed coverage over time at each site plotted as a function of turfgrass cover showed a strong negative trend with more turfgrass cover resulting in less weed cover (Fig 2.4); likewise, more bare soil cover resulted in more weed coverage up to 50%, but then decreased, since our coverage is constrained between 0-1 (Fig. 2.4). Weed cover in field plots showed little to no trend as a function of the seed bank seedling density (Fig. 2.5), Chao estimated species richness (Fig. 2.6), and observed species density (Fig. 2.6). Seed bank characteristics not having a significant impact on weed coverage is not surprising, since it is well documented that the seed bank characteristics usually do not accurately reflect the concurrently growing aboveground vegetation at a site (Bekker et al., 1997; D. P. Coffin & Lauenroth, 1989; Pekas & Schupp, 2013; Skowronek et al., 2014; Thompson, 1986; Thompson & Grime, 1979). We were also surprised that observed seed bank species density was included in the final stepwise model rather than Chao estimated seed bank species richness, thought to be a closer approximation to the actual number of species at each site.

The results of the NMDS suggest that there is a significant difference in roadside seed bank composition between NNE and SSW sites. From examining the NMDS, rush species are on the far left side and crabgrass (*Digitaria* spp.) on the right (Fig. 2.3A,B), so we hypothesize the interaction between warmer climate normal air temperatures (Table 2.8) is likely causing the separation with greater abundance of warm-season annual species in warmer sunny areas of the state. The abundance of large crabgrass was very high at Marshall; this site was located adjacent to the golf course and was maintained regularly with above-average mowing intervals and lower than average heights of cut for roadside vegetation, conditions known to favor crabgrass abundance (Davis, 1958). Additionally, Marshall contained 12 observed species, one of the lowest, which also may have been due to intensive mowing, since it has been shown that increasing management intensity reduces species richness and increases seed bank seedling density (Auestad et al., 2013; Dölle & Schmidt, 2009).

Several limitations may have influenced the results of this study. Different research sites were affected differently by abiotic factors. For example, Brainerd and Bemidji contained low turfgrass coverage, resulting in high weed and/or bare soil coverage relative to other research sites; therefore, the seed bank characteristics were clearly not a factor. We also found differences in bulk density at our field sites which could have lessened weed seedling emergence and coverage (Stark et al., 2003). Additionally, we only tested 1800 grams of soil for each research site and the Chao estimated species richness shows that sampling limitations exist, therefore, we may have only characterized half or less of the species at different sites. At the International Falls site, we visually estimated there could be upwards of 100 species total of rushes, sedges, and grasses (*Poaceae*), along with various broadleaf plants growing in a small 0.2-hectare area. So, roadside aboveground vegetation has the capacity to be quite diverse even within a single research site. Comparing the seed bank species differences across different seeding years too closely could also be misleading, since sampling was different, and sampling methods consisted of soil collected from too few cores. Sampling limitations are a common factor impacting most seed bank studies (Thompson et al., 1997).

In general, only 3-6% of the total number of viable seeds germinate in the aboveground vegetation as seedlings, assuming adequate moisture (Roberts & Ricketts, 1979), and so testing the seed bank density characteristics of a site as a covariate may have limitations if more or less species are germinating than normal in the greenhouse compared to field soil. Previous roadside turfgrass research has found that seeding cool-season mixtures in late summer to early fall is ideal in Minnesota because soil temperatures are high and weed pressure is low (Watkins & Trappe, 2017), and it is known that the timing of cultivation affects species abundance (Roberts & Ricketts, 1979). We therefore hypothesize if these sites were seeded in early summer, then seed bank characteristics could have likely played a more significant and long-lasting effect.

We conclude that roadsides that are regularly mowed in Minnesota contain mostly undesirable ruderal vegetation in the seed bank, but greater richness of native species than we expected. The properties of a seed bank are not preeminent factors impacting weed coverage over time, since we found that the most significant factor to reduce weed coverage is to maintain adequate seeded turfgrass coverage over time.

For future testing of roadside seed banks, we recommend utilizing improved testing procedures (Heerdt et al., 1996) and sampling procedures (Bigwood & Inouye, 1988; Thompson et al., 1997; Warr et al., 1993), and to thoroughly characterize the aboveground vegetation growing at a site to better understand the species dominating a site. Identifying how certain functional groups in the seed bank may impact the type of seeded coverage over time could be tested and a mixture could be developed with greater compatibility with the type and abundance of seed bank vegetation.

Table 2.1: Soil characteristics for each block within the fourteen research sites. The University of Minnesota Soil Testing Laboratory analyzed all samples using standard methods.

| Research site | Block | Bray P[c] | Olsen P[c] | K[c] | OM | pH | Sat. elect. conductivity (mmhos/cm)[b] | Sand (%) | Silt (%) | Clay (%) | Soil textural class[a] | Fe[bc] | Mn[bc] | Zn[bc] | Cu[bc] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bemidji | 1 | 33 | NA | 74 | 2.0 | 7.3 | 1.0 | 70.0 | 10.0 | 20.0 | SCL-SL | 17.27 | 5.40 | 3.30 | 0.37 |
| Bemidji | 2 | 32 | NA | 64 | 1.7 | 7.3 | 1.1 | 70.0 | 11.3 | 18.8 | SL | 17.03 | 5.63 | 2.34 | 0.34 |
| Bemidji | 3 | 50 | NA | 108 | 2.5 | 7.3 | 1.1 | 68.7 | 10.0 | 21.3 | SCL | 23.30 | 5.29 | 2.88 | 0.32 |
| Brainerd | 1 | 31 | 9 | 82 | 3.30 | 7.4 | 0.9 | 70 | 9 | 21 | SCL | 56.64 | 5.21 | 42.45 | 10.34 |
| Brainerd | 2 | 25 | 7 | 64 | 3.10 | 7.4 | 0.7 | 71 | 8 | 21 | SCL | 76.27 | 4.47 | 46.33 | 14.17 |
| Brainerd | 3 | 30 | 11 | 89 | 2.90 | 7.4 | 0.6 | 73 | 5 | 23 | SCL | 42.48 | 4.34 | 41.08 | 18.55 |
| Chatfield | 1 | 26 | 14 | 166 | 3.10 | 7.4 | 0.6 | 50 | 18 | 33 | SCL | 27.59 | 8.30 | 1.39 | 0.43 |
| Chatfield | 2 | 26 | 13 | 117 | 2.50 | 7.5 | 0.8 | 53 | 16 | 31 | SCL | 23.91 | 8.06 | 1.64 | 0.40 |
| Chatfield | 3 | 25 | 9 | 116 | 2.50 | 7.6 | 0.9 | 55 | 14 | 31 | SCL | 17.48 | 8.16 | 1.26 | 0.30 |
| Duluth | 1 | 7 | 4 | 56 | 1.7 | 7.5 | 1.4 | 52.5 | 25.0 | 22.5 | SCL | 53.65 | 8.74 | 2.08 | 4.73 |
| Duluth | 2 | 11 | 5 | 49 | 2.6 | 7.5 | 1.5 | 53.8 | 23.7 | 22.5 | SCL | 54.59 | 5.73 | 2.33 | 4.71 |
| Duluth | 3 | 12 | 5 | 63 | 3.5 | 7.5 | 2.0 | 58.8 | 20.0 | 21.2 | SCL | 62.97 | 4.20 | 6.81 | 4.66 |
| E. G. Forks | 1 | 0 | 14 | 297 | 5.20 | 7.8 | 0.7 | 5 | 49 | 46 | Silty clay | 10.12 | 5.84 | 1.92 | 1.36 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E. G. Forks | 2 | 0 | 8 | 226 | 5.00 | 7.9 | 0.6 | 4 | 49 | 48 | Silty clay | 10.48 | 6.81 | 1.64 | 1.42 |
| E. G. Forks | 3 | 0 | 9 | 196 | 4.70 | 7.9 | 0.6 | 3 | 48 | 49 | Silty clay | 9.83 | 6.87 | 1.57 | 1.43 |
| Edina | 1 | 30 | NA | 89 | 5.6 | 7.2 | 1.3 | 51.3 | 23.8 | 25.0 | SCL | 42.43 | 4.21 | 5.96 | 1.01 |
| Edina | 2 | 23 | NA | 64 | 6.0 | 7.2 | 1.6 | 47.5 | 26.2 | 26.2 | SCL | 40.06 | 4.61 | 6.56 | 1.09 |
| Edina | 3 | 25 | NA | 56 | 5.8 | 7.2 | 1.4 | 48.8 | 26.2 | 25.0 | SCL | 40.47 | 5.25 | 7.70 | 1.22 |
| Fergus Falls | 1 | 11 | 5 | 264 | 5.20 | 7.9 | 1.1 | 48 | 21 | 31 | SCL | 14.82 | 5.12 | 6.37 | 1.19 |
| Fergus Falls | 2 | 20 | 10 | 218 | 5.30 | 7.9 | 1.0 | 51 | 19 | 30 | SCL | 12.54 | 3.87 | 6.32 | 1.15 |
| Fergus Falls | 3 | 16 | 7 | 210 | 4.80 | 7.9 | 0.8 | 52 | 19 | 29 | SCL | 11.92 | 4.63 | 5.83 | 1.12 |
| G. Rapids | 1 | 39 | 12 | 61 | 1.80 | 7.4 | 1.0 | 59 | 19 | 23 | SCL | 49.00 | 7.25 | 2.76 | 0.85 |
| G. Rapids | 2 | 36 | 9 | 56 | 1.50 | 7.4 | 0.8 | 64 | 15 | 21 | SCL | 41.93 | 7.01 | 2.47 | 1.07 |
| G. Rapids | 3 | 40 | 11 | 61 | 2.20 | 7.5 | 0.6 | 58 | 21 | 21 | SCL | 38.05 | 7.11 | 3.62 | 1.17 |
| Int. Falls | 1 | 4 | 4 | 145 | 5.7 | 7.5 | 1.3 | 38.7 | 21.3 | 40.1 | Clay | 42.03 | 2.54 | 2.68 | 1.27 |
| Int. Falls | 2 | 5 | 4 | 146 | 5.3 | 7.6 | 0.7 | 34.9 | 26.3 | 38.8 | Clay loam | 30.78 | 1.74 | 2.69 | 1.08 |
| Int. Falls | 3 | 5 | 4 | 97 | 8.9 | 7.5 | 1.3 | 50.0 | 18.8 | 31.3 | SCL | 47.20 | 1.80 | 5.09 | 1.21 |
| Marshall | 1 | 10 | 15 | 249 | 4.00 | 7.6 | 0.4 | 35 | 24 | 41 | Clay | 21.82 | 8.04 | 3.22 | 1.10 |
| Marshall | 2 | 7 | 10 | 175 | 3.80 | 7.8 | 0.5 | 34 | 24 | 43 | Clay | 19.84 | 7.43 | 3.73 | 1.19 |
| Marshall | 3 | 9 | 8 | 186 | 4.20 | 7.9 | 0.5 | 34 | 23 | 43 | Clay | 19.09 | 7.39 | 3.28 | 1.27 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Roseville | 1 | 14.5 | 6.5 | 65 | 6.10 | 7.9 | 0.7 | 60 | 15 | 25 | SCL | 42.95 | 3.95 | 15.80 | 2.63 |
| Roseville | 2 | 13 | 6 | 87 | 4.60 | 8.0 | 0.6 | 69 | 6 | 25 | SCL | 25.18 | 3.96 | 11.89 | 2.59 |
| Roseville | 3 | 16 | 6 | 71 | 4.70 | 7.8 | 0.6 | 56 | 19 | 25 | SCL | 26.00 | 3.75 | 10.76 | 2.54 |
| Saint Cloud | 1 | 40 | NA | 139 | 3.1 | 6.4 | 1.5 | 51.2 | 20.0 | 28.8 | SCL | 116.36 | 10.91 | 3.53 | 0.75 |
| Saint Cloud | 2 | 42 | NA | 135 | 2.6 | 7.0 | 1.2 | 58.7 | 15.0 | 26.3 | SCL | 47.56 | 7.80 | 1.88 | 0.49 |
| Saint Cloud | 3 | 58 | NA | 153 | 2.4 | 7.2 | 1.5 | 65.0 | 11.3 | 23.8 | SCL | 29.15 | 6.40 | 1.85 | 0.36 |
| Willmar | 1 | 2 | 9 | 166 | 3.6 | 7.5 | 1.7 | 46.3 | 23.7 | 30.0 | SCL | 17.44 | 4.35 | 9.24 | 1.21 |
| Willmar | 2 | 2 | 8 | 181 | 3.4 | 7.5 | 1.5 | 37.5 | 30.0 | 32.5 | Clay loam | 15.64 | 3.86 | 3.91 | 0.88 |
| Willmar | 3 | 5 | NA | 202 | 3.3 | 7.4 | 1.4 | 43.7 | 25.0 | 31.3 | Clay loam | 20.13 | 4.11 | 4.76 | 0.97 |
| Worthington | 1 | 26 | NA | 166 | 5.1 | 7.2 | 1.5 | 20.0 | 36.3 | 43.8 | Clay | 26.91 | 7.18 | 2.83 | 0.86 |
| Worthington | 2 | 18 | NA | 161 | 4.8 | 7.4 | 0.4 | 16.3 | 38.7 | 45.0 | Clay | 29.29 | 7.59 | 2.75 | 1.15 |
| Worthington | 3 | 19 | NA | 183 | 5.2 | 7.3 | 0.6 | 12.5 | 43.7 | 43.7 | Silty clay | 30.98 | 7.75 | 3.07 | 1.03 |

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[a] SCL = Sandy clay loam, SL= Sandy loam,

[b] An additional 20 cores of soil samples were collected and composited within each block at each research sites seeded in 2018 between Jun.-Aug. 2020 and were tested for heavy metals and saturated paste extract electrical conductivity.

[c] Units of mg $kg^{-1}$

Table 2.2: Emerged seedlings within each subsample of each block at each research site. A total of 1,375 seedlings emerged from the seed bank analysis in this study from both seeding years; 826 from sites seeded in 2018 and 549 from sites seeded in 2019. Sampling and preparation methods varied slightly between seeding years.

| | | Block | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | | 2 | | | 3 | | |
| Research site | Seeding year | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Brainerd | 2018 | 19 | 5 | 7 | 3 | 8 | 5 | 16 | 0 | 2 |
| Chatfield | 2018 | 2 | 5 | 10 | 4 | 3 | 12 | 1 | 5 | 10 |
| East Grand Forks | 2018 | 7 | 5 | 2 | 5 | 2 | 3 | 4 | 3 | 6 |
| Fergus Falls | 2018 | 9 | 11 | 11 | 6 | 4 | 10 | 12 | 4 | 8 |
| Grand Rapids | 2018 | 1 | 10 | 3 | 3 | 7 | 2 | 3 | 7 | 4 |
| Marshall | 2018 | 39 | 54 | 5 | 69 | 65 | 3 | 44 | 33 | 6 |
| Roseville | 2018 | 42 | 34 | 24 | 27 | 13 | 18 | 26 | 34 | 21 |
| Bemidji | 2019 | 16 | 13 | 10 | 18 | 8 | 12 | 15 | 9 | 13 |
| Duluth | 2019 | 2 | 4 | 4 | 3 | 9 | 9 | 2 | 8 | 6 |
| Edina | 2019 | 4 | 12 | 2 | 3 | 8 | 6 | 0 | 3 | 4 |
| Int. Falls | 2019 | 21 | 7 | 3 | 6 | 9 | 0 | 15 | 13 | 3 |
| Saint Cloud | 2019 | 8 | 16 | 14 | 14 | 18 | 11 | 8 | 7 | 13 |
| Willmar | 2019 | 2 | 9 | 5 | 6 | 10 | 7 | 4 | 5 | 3 |
| Worthington | 2019 | 16 | 7 | 15 | 14 | 5 | 19 | 13 | 3 | 17 |

Figure 2.1: Map showing fourteen research sites that were seeded in the state of Minnesota. The darker thicker boundaries distinguish the 8 MnDOT regions and the thinner boundaries represent county borders.

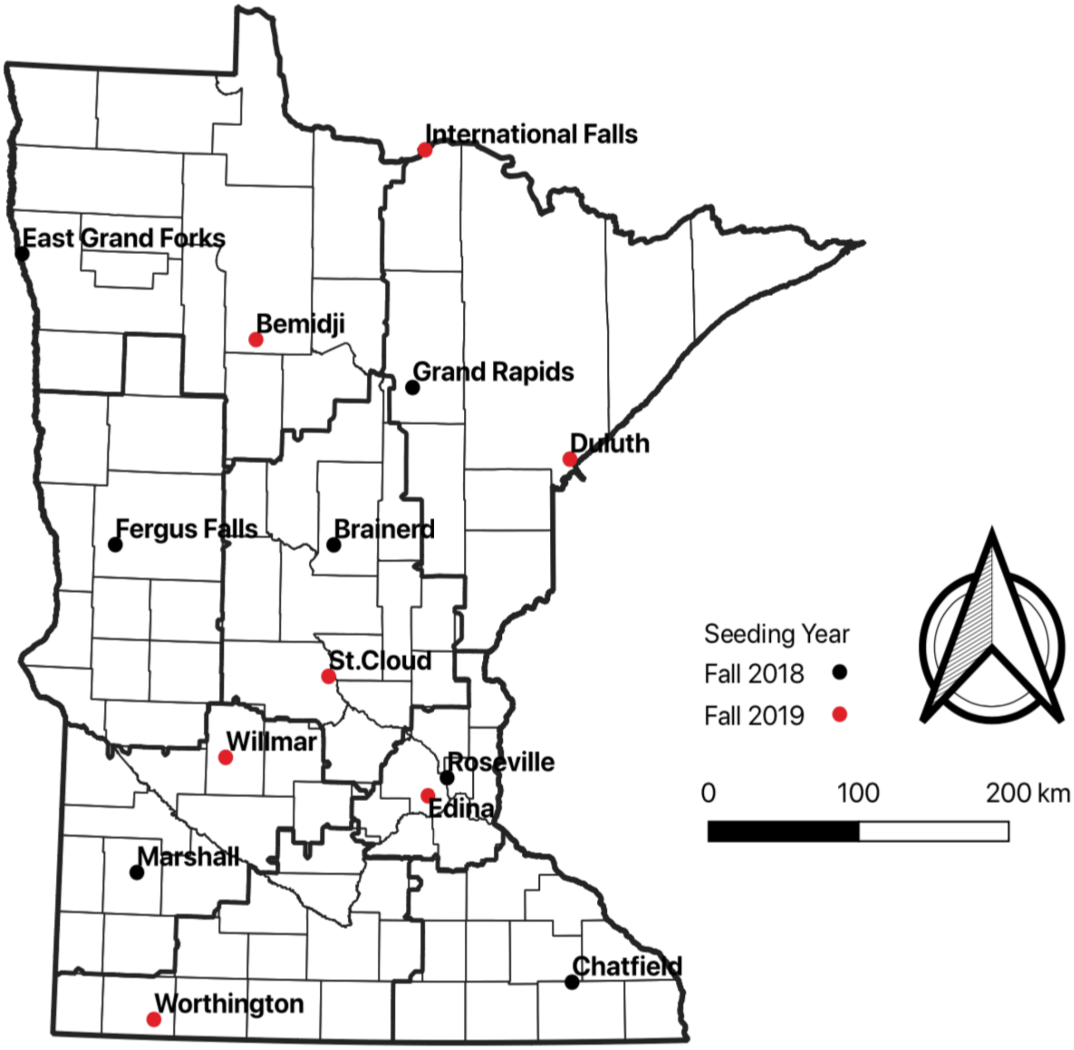

Table 2.3: The total number of seedlings, observed species, and chao estimated species that emerged from seed bank soil at each research site. Chao estimated species richness is based on the number of seedlings within a species at a research site that emerged once or twice.

| | | Species richness | | Evenness |
|---|---|---|---|---|
| Research site | Total emerged seedlings | Observed | Chao estimated | PIE[a] |
| Bemidji | 114 | 20 | 40 | 0.85 |
| Brainerd | 65 | 20 | 24 | 0.89 |
| Chatfield | 52 | 17 | 42 | 0.86 |
| Duluth | 47 | 16 | 22 | 0.92 |
| East Grand Forks | 37 | 12 | 14 | 0.83 |
| Edina | 42 | 10 | 34 | 0.57 |
| Fergus Falls | 75 | 13 | 29 | 0.67 |
| Grand Rapids | 40 | 10 | 34 | 0.60 |
| International Falls | 77 | 15 | 40 | 0.83 |
| Marshall | 318 | 12 | 30 | 0.22 |
| Roseville | 239 | 21 | 29 | 0.55 |
| Saint Cloud | 109 | 20 | 30 | 0.87 |
| Willmar | 51 | 19 | 36 | 0.89 |
| Worthington | 109 | 18 | 22 | 0.80 |

[a] Probability of an interspecific encounter (PIE) ranges from 0-1 to identify the evenness within a research site (e.g. 1=each individual seedling is a unique species, 0=each individual seedling is the same species).

Table 2.4: Density estimates for average total soil bulk, seedling and observed species richness, and chao estimated species richness from the soil seed bank from each research site.

| | Density | | | |
|---|---|---|---|---|
| Research site | Soil bulk[a] (g cm$^{-3}$) | Seedling[b] (seedlings L$^{-1}$) | Obs. species[c] (species L$^{-1}$) | Chao estimated[d] (species L$^{-1}$) |
| Bemidji | 1.43 | 90.87 | 15.94 | 31.88 |
| Brainerd | 1.40 | 50.56 | 15.56 | 18.67 |
| Chatfield | 1.28 | 36.88 | 12.06 | 29.78 |
| Duluth | 1.61 | 41.92 | 14.27 | 19.62 |
| E. G. Forks | 1.14 | 23.42 | 7.59 | 8.86 |
| Edina | 1.35 | 31.49 | 7.50 | 25.49 |
| Fergus Falls | 1.28 | 53.14 | 9.21 | 20.55 |
| G. Rapids | 1.71 | 38.10 | 9.53 | 32.39 |
| Int. Falls | 1.05 | 44.89 | 8.74 | 23.32 |
| Marshall | 1.18 | 208.51 | 7.87 | 19.67 |
| Roseville | 1.27 | 168.89 | 14.84 | 20.49 |
| Saint Cloud | 1.43 | 86.39 | 15.85 | 23.78 |
| Willmar | 1.36 | 38.57 | 14.37 | 27.23 |
| Worthington | 1.11 | 67.15 | 11.09 | 13.55 |

[a] Total coarse bulk density includes unsieved coarse fragments.

[b] Density (seedlings L$^{-1}$) was calculated by taking the total number of seedlings at each research site and dividing that by 1800 g (for that was the total amount of weight tested at each site then multiplying that value by the mean total bulk density at that site and then multiplying by 1000 to result in seedlings L$^{-1}$.

[c] Observed species density was calculated by taking the number of observed species at each research site divided by the total number of seedlings and then multiplying that value by the density in seedlings L$^{-1}$.

[d] Chao estimated species density was calculated by taking the Chao species richness for each site (Chao, 1984) and then dividing that value by the total number of seedlings and then multiplying that value by the density in seedlings $L^{-1}$.

Table 2.5: Number of seedlings and observed species at each site from the soil seed bank classified by origin. Seedlings classified as cryptic refers to specimens that do not have a clear origin or specimens that were not identified to the species level (i.e. the genus level contains native and non-native species), or samples that died before they could be identified.

| | Species origin | | | | | |
|---|---|---|---|---|---|---|
| | Native | | Non-native | | Cryptic | |
| Research site | Count | Species richness | Count | Species richness | Count | Species richness |
| Bemidji | 0.28 | 0.50 | 0.65 | 0.45 | 0.07 | 0.05 |
| Brainerd | 0.34 | 0.50 | 0.23 | 0.25 | 0.43 | 0.25 |
| Chatfield | 0.17 | 0.41 | 0.67 | 0.47 | 0.15 | 0.12 |
| Duluth | 0.32 | 0.38 | 0.49 | 0.38 | 0.19 | 0.25 |
| E. G. Forks | 0.46 | 0.25 | 0.38 | 0.50 | 0.16 | 0.25 |
| Edina | 0.17 | 0.60 | 0.83 | 0.40 | 0.00 | 0.00 |
| Fergus Falls | 0.07 | 0.31 | 0.64 | 0.62 | 0.29 | 0.08 |
| Grand Rapids | 0.10 | 0.30 | 0.88 | 0.60 | 0.03 | 0.10 |
| Int. Falls | 0.35 | 0.53 | 0.40 | 0.27 | 0.25 | 0.20 |
| Marshall | 0.03 | 0.33 | 0.92 | 0.50 | 0.06 | 0.17 |
| Roseville | 0.10 | 0.38 | 0.88 | 0.52 | 0.02 | 0.10 |
| Saint Cloud | 0.24 | 0.45 | 0.76 | 0.55 | 0.00 | 0.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Willmar | 0.24 | 0.26 | 0.76 | 0.74 | 0.00 | 0.00 |
| Worthington | 0.17 | 0.44 | 0.82 | 0.50 | 0.02 | 0.06 |
| Average[a] | 0.17 | 0.41 | 0.74 | 0.48 | 0.09 | 0.11 |

[a] Not the average calculated from the proportions listed in this table, but the average incorporating all seedlings.

Figure 2.2: Proportion of the count of the emerged seedlings and the number of observed species at each research site classified by life cycle. Unknown represents an emerged seedling that died before it could be identified. Carpet vervain and common dandelion (*Taraxacum officinale* G.H. Weber) were classified as weak perennial.

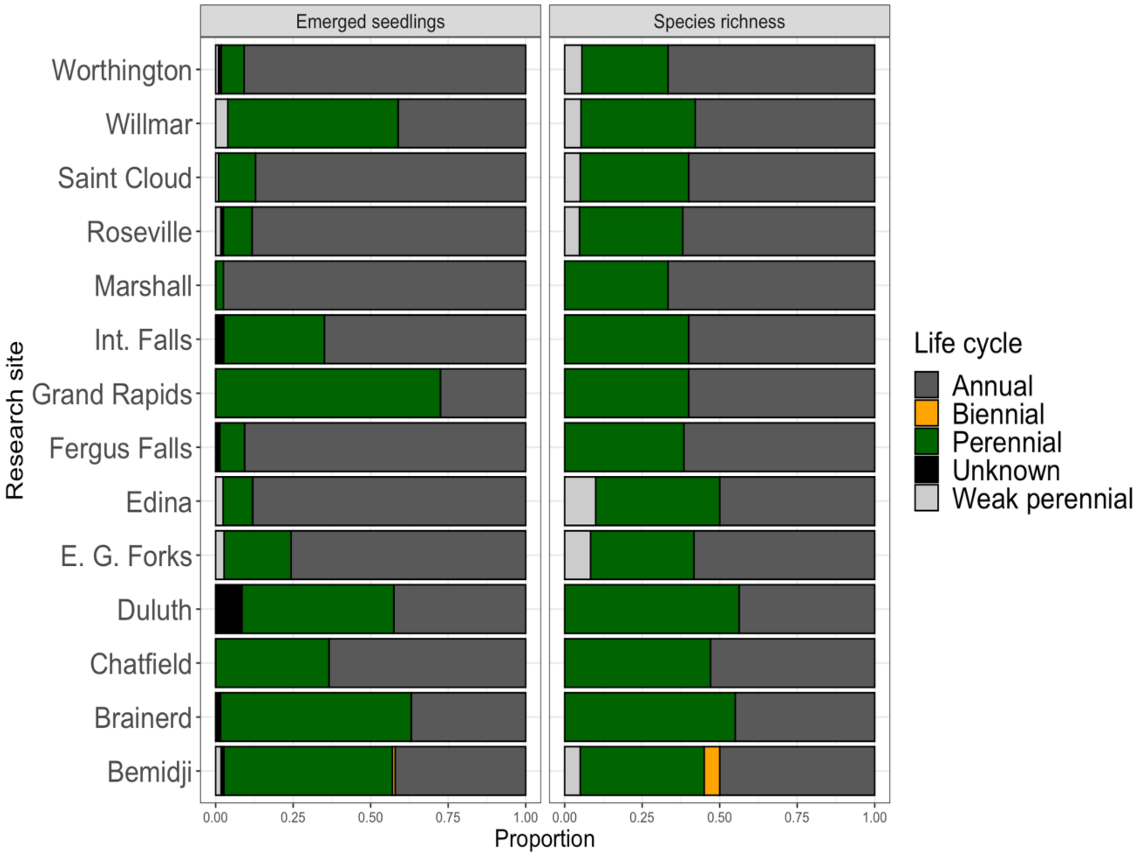

Figure 2.3: NMDS ordination plot of different vascular plants identified to at least the genus level (A) and north-south separation in species composition based on PERMANOVA results (Stress=0.199) (B). Labels in (A) are the first five letters of the genus followed by the first three letters of the species name, and inconspicuous circles behind some labels indicate there are multiple species in that same ordination space. Labels in (B) correspond to different research sites (Bem=Bemidji, Bra=Brainerd, Cha=Chatfield, Dul=Duluth, Eas=East Grand Forks, Edi=Edina, Fer=Fergus Falls, Gra=Grand Rapids, Int=International Falls, Mar=Marshall, Ros=Roseville, Sai=Saint Cloud, Wil=Willmar, and Wor=Worthington).

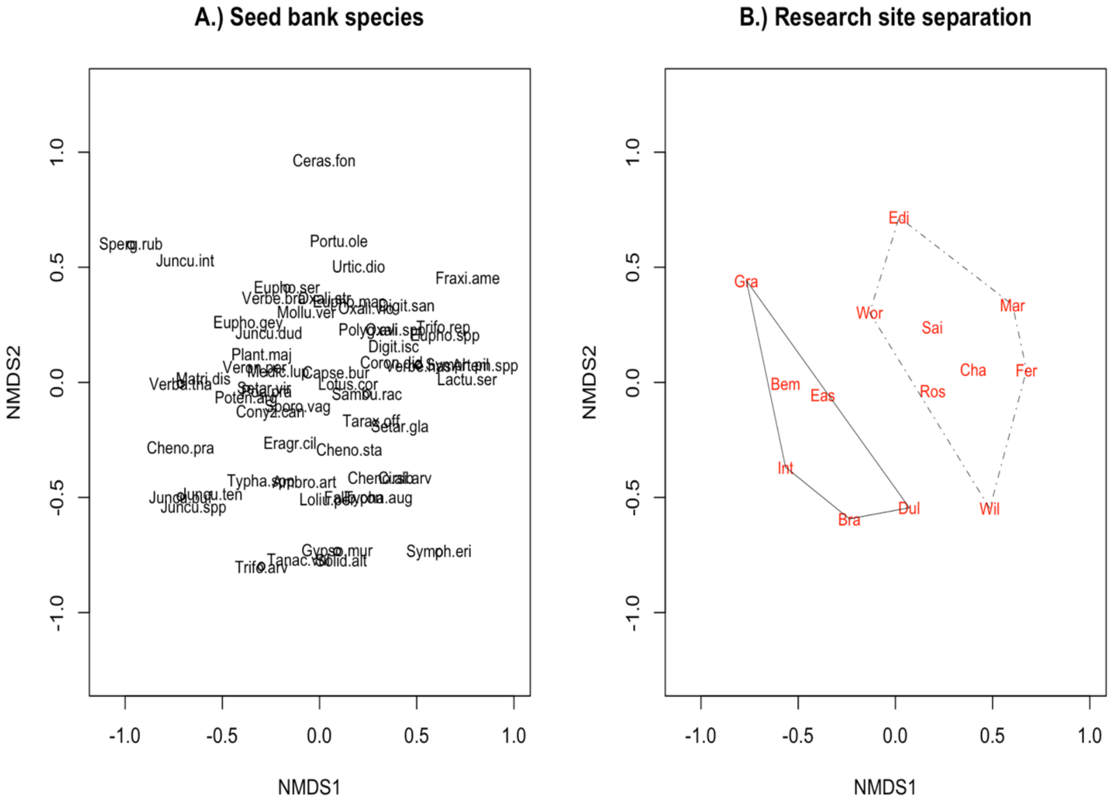

Table 2.6: Generalized linear model summary results with weed coverage (%) as the response variable. Seeding year coefficient distinguishes the research sites seeded in separate seeding years. Time represents the order of vegetation sampling that each site was sampled (sites seeded in 2018 have a total of 5 sampling instances and sites seeded in 2019 have a total of 4 sampling instances). All three coverage variables (weed, turfgrass and bare soil) are a proportion bounded from 0-1. Observed seed bank species density is in units of count per liter.

| Coefficients | Estimate | SE | z value | Pr(>\|z\|) |
|---|---|---|---|---|
| (Intercept) | 1.59152 | 0.54935 | 2.897 | 0.00377 |
| Seeding year 2019 | -0.70069 | 0.29845 | -2.348 | 0.01889 |
| Time | 0.43612 | 0.13895 | 3.139 | 0.0017 |
| Turfgrass coverage | -6.39802 | 0.20146 | -31.758 | <2E-16 |
| Bare soil coverage | -6.57867 | 0.27494 | -23.928 | <2E-16 |
| Observed seed bank species density | 0.10011 | 0.04162 | 2.405 | 0.01617 |
| Time:Observed seed bank species density | -0.02552 | 0.01141 | -2.236 | 0.02533 |
| Seeding year 2019:Time | 0.22727 | 0.08888 | 2.557 | 0.01056 |

Null deviance: 2312.3 on 7559 degrees of freedom. Residual deviance: 245.4 on 7552 degrees of freedom. McFadden pseudo $R^2$ value = 0.89.

Table 2.7: Generalized linear model deviance results from Table 2.6 above. Seeding year coefficient distinguishes the research sites seeded in separate seeding years. Time represents the order of vegetation sampling that each site was sampled (sites seeded in 2018 have a total of 5 sampling instances and sites seeded in 2019 have a total of 4 sampling instances). Turfgrass and bare soil coverage are a proportion bounded from 0-1. Observed seed bank species density is in units of count per liter. Resid. Df = residual degrees of freedom. Resid. Dev = residual deviance.

| Coefficients | Df | Deviance | Resid. Df | Resid. Dev |
|---|---|---|---|---|
| (Intercept) | NA | NA | 7559 | 2312 |
| Seeding year 2019 | 1 | 82.2 | 7558 | 2230 |
| Time | 1 | 488 | 7557 | 1742 |
| Turfgrass coverage | 1 | 736 | 7556 | 1006 |
| Bare soil coverage | 1 | 750 | 7555 | 256 |
| Observed seed bank species density | 1 | 0.81 | 7554 | 255 |
| Time:Observed seed bank species density | 1 | 3.15 | 7553 | 252 |
| Seeding year 2019:Time | 1 | 6.73 | 7552 | 245 |

Figure 2.4: Mean weed coverage as a function of turfgrass and bare soil coverage averaged over all sampling times within each research sites. Seeding year is shown faceted in both graphs. The three coverage variables (weed, turf, and bare soil) changed over time at these different research sites, so there are limitations to viewing this graph as an average.

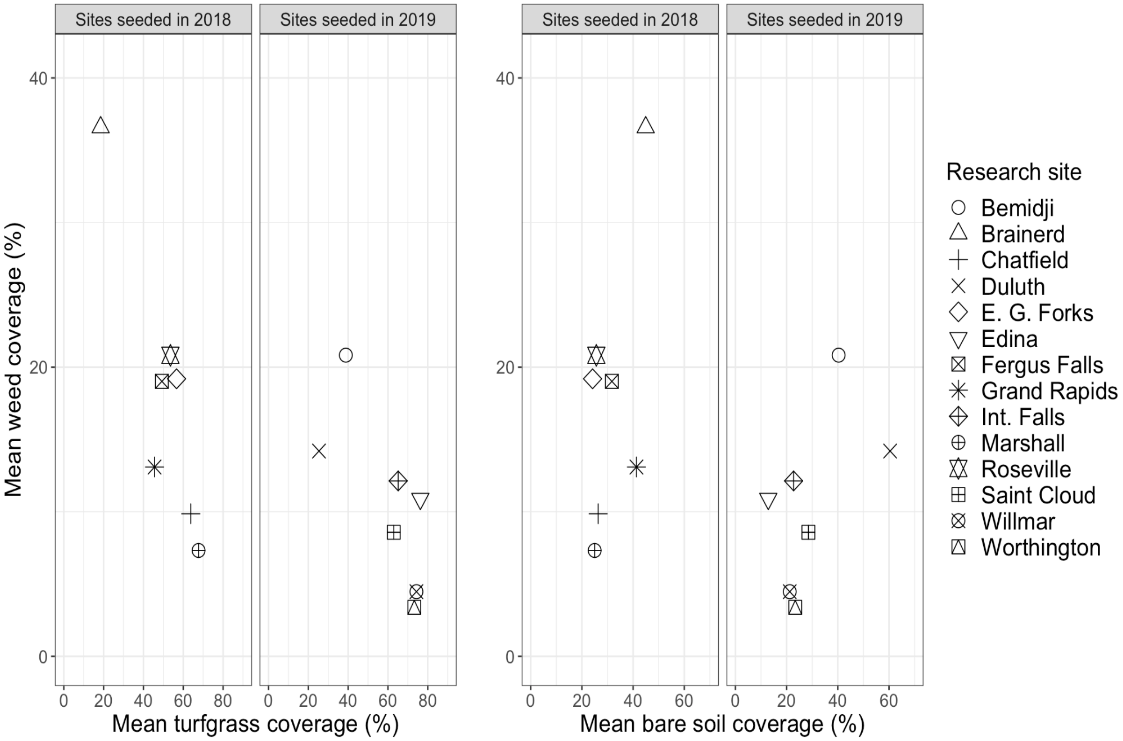

Figure 2.5: Mean weed coverage as a function of seedling density at each research site. Mean weed cover is an average across all sampling instances. The weed coverage changed over time at these different research sites, so there are limitations to viewing this graph as an average.

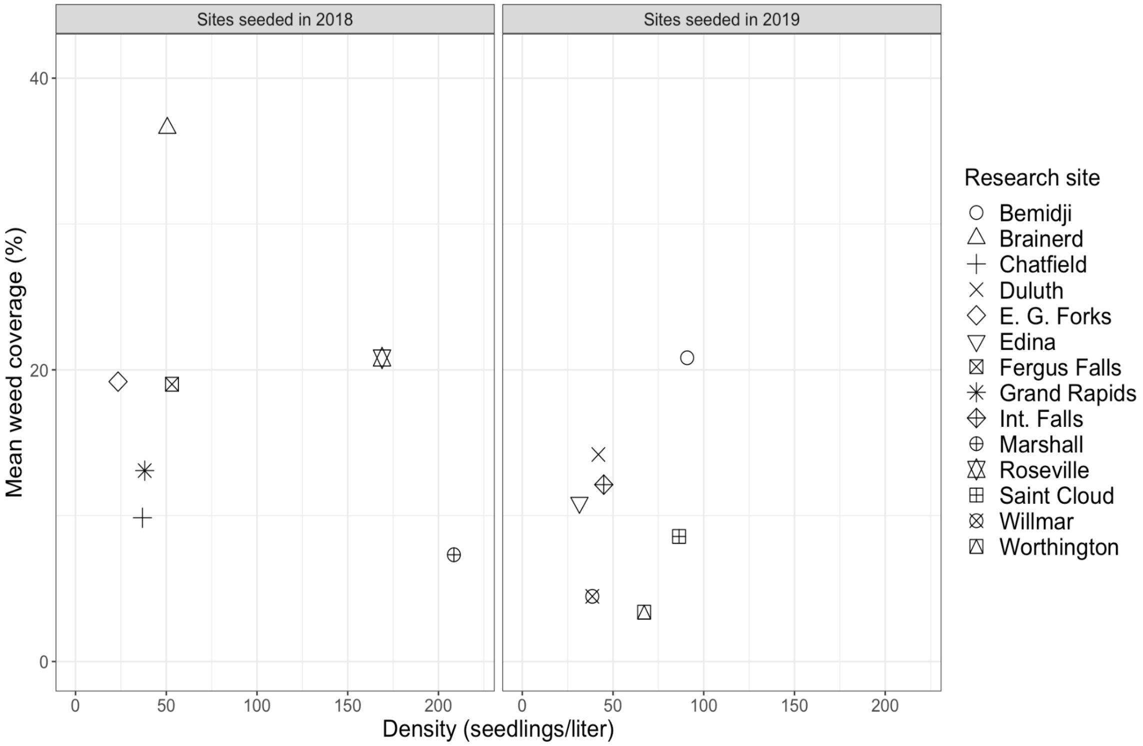

Figure 2.6: Mean weed coverage as a function of Chao estimated and observed species density at each research site. The weed coverage changed over time at these different research sites, so there are limitations to viewing this graph as an average.

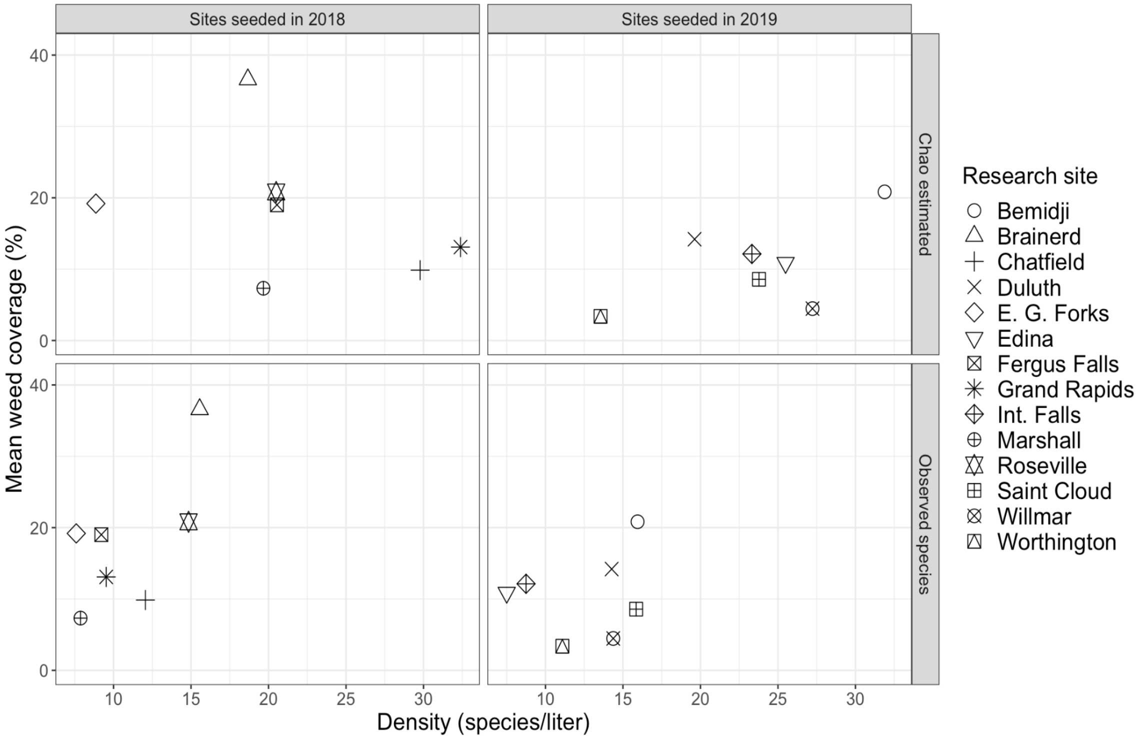

Table 2.8: Climate normal (1991-2020) weather data for all 14 research sites from the nearest station containing the most complete dataset. TMAX = temperature maximum, TMIN = temperature minimum, PRCP = rainfall precipitation, SNOW = snowfall precipitation.

| Research site | TMAX[a] ºC | TMIN[a] ºC | PRCP[b] (mm) | SNOW[b] (mm) |
|---|---|---|---|---|
| Bemidji | 10.28 | -0.97 | 689.82 | 1194.13 |
| Brainerd | 11.26 | -1.06 | 772.19 | 1223.59 |
| Chatfield | 12.18 | 2.09 | 880.83 | 1341.87 |
| Duluth | 9.52 | -0.44 | 792.07 | 2291.96 |
| East Grand Forks | 10.43 | -0.51 | 576.02 | 1264.55 |
| Edina | 12.97 | 3.30 | 802.62 | 1302.83 |
| Fergus Falls | 10.45 | -1.18 | 598.96 | 493.48 |
| Grand Rapids | 9.84 | -1.49 | 698.98 | 1087.06 |
| International Falls | 9.41 | -2.97 | 644.25 | 1855.52 |
| Marshall | 13.25 | 1.98 | 734.43 | 1149.52 |
| Roseville | 12.87 | 2.79 | 835.42 | 731.58 |
| Saint Cloud | 11.97 | 0.37 | 723.50 | 1231.54 |
| Willmar | 11.45 | 0.54 | 775.01 | 1204.22 |
| Worthington | 12.52 | 1.34 | 783.05 | 1190.62 |

[a] Average of average monthly climate normals.

[b] Sum of average monthly precipitation.

# Chapter 3

# The Effects of Greater Seeded Turfgrass Diversity for Roadsides across Minnesota

## Summary

Roadsides are often planted with turfgrass seed mixtures. Designing these mixtures to withstand salting, ice encasement, temperature and moisture extremes, snowplow damage, and poor maintenance is difficult. Our objective was to determine the effect of seeded turfgrass species richness on ground cover over time alongside roadsides in diverse regions in Minnesota. The experiment tested 6 turfgrass species, each represented by a single cultivar, in monocultures, pairwise interactions, some three-way mixtures, and a single six-way mixture at 7 sites seeded in the fall of 2018 and 7 in the fall of 2019. Each site was a randomized complete block design consisting of 40 treatments with 3 blocks and each plot consisted of an area of 2.3 $m^2$ along the curb. The plant species and total ground cover was collected at each site using the quadrat-grid intersection method over two growing seasons. Turfgrass, weed, and bare soil coverage was compared among treatments using a generalized linear mixed effects model. We found a highly significant interaction between time, defined as the order of vegetation sampling, and species richness for turfgrass coverage ($P < 0.001$); illustrating that there was more coverage, and that effect increased over time, as more species were added to a mixture. Each additional time increment also found more weed coverage (Est=0.88, $p<0.001$) and less bare soil coverage (Est=-1.14, $p<0.001$). Spatial stability of turfgrass cover was greater with increasing species richness ($P<0.001$). Our findings show that greater species richness is important to increasing and stabilizing roadside turfgrass coverage.

## Introduction

Roadsides contain at least 1% of the land area of most states in the United States and can affect species, soil, and water covering approximately 20% of the country (Forman, 2000). In Minnesota, there is at least 20,000 hectares of regularly mowed boulevard roadside vegetation. Studying the use of turfgrass species on roadsides can have a significant economic and ecological impact in these areas.

Seeding turfgrass species on roadsides largely began with the development of the interstate system in the late 1920s and then continued with interstates later being modeled after the Autobahn in Germany (Weingroff, 2013). The intended function was to provide adequate soil coverage to reduce erosion and to provide a uniform aesthetic while keeping maintenance costs low (Boeker, 1970; Duell & Schmit, 1975; Hottenstein, 1969; White & Smithberg, 1972). Turfgrass species naturally fulfill the aesthetic and visibility criteria since they tolerate mowing due to their short-stature growth habit, which maintains visibility for drivers. Many also have a rhizomatous and stoloniferous growth habit allowing them to easily spread after disturbances (Beard, 1973). Most turfgrass species are also well known, relatively easy to establish, and cost efficient to seed and maintain compared to other species when considering the large planting areas. Although, maintaining adequate coverage along roadsides over time has been difficult to achieve, especially in the first 3 m adjacent to roads due to various abiotic stresses including salts from deicing operations, ice encasement, drought, heat, and poor soils (Hopkinson et al., 2016); as a result, these areas often contain significant quantities of annuals and other poorly desired vegetation (Biesboer et al., 1998). In Minnesota, roadside vegetation is considered adequate once 70% coverage is achieved with no long-term evaluation [Minn. R. 7090 section 13.2], and if these areas are reseeded due to establishment failure, the economic cost is high.

Broader functions for roadsides such as to preserve and promote ecological diversity (Williams et al., 2015) have gained more attention in recent decades. It is important to recognize that narrower functions such as maintaining coverage and visibility and broader functions such as pollinator habitat, ecological diversity, and rooting heterogeneity (Brown et al., 2010) are not necessarily competing objectives. For instance, Hector & Bagchi (2007) found that when more species are added to a natural ecosystem, it will provide or fulfill more functions. The question of whether diversity increases multifunctionality in managed roadside turfgrass is a relatively unexplored topic, but would likely increase, unless if tall-stature species compromise visibility for drivers or greater habitat causes more roadkill. If the net ecological impact is considered on the surrounding environment, then a roadside providing greater functionality may have far-reaching effects (A. Coffin, 2007).

Turfgrass researchers have and continue to focus on the performance of monocultures to determine seed mixture components; the implication being that if a turfgrass species does not perform at least moderately in a monoculture setting, then it will not be adequate as a component in a mixture. This assumption has potential limitations because several experiments have shown that monoculture performance does not necessarily dictate a species' performance in a mixture (Knott & Mundt, 1990; Lopez & Mundt, 2000), and the inclusion of some species that perform below average as monocultures may still benefit the overall performance of a mixture (Robins & Bushman, 2020). Yet, it is important to know which species and cultivars are at least relatively adapted to roadsides, because some can be identified as excellent candidates (Engelhardt, 2016), but perform poorly in field experiments. For example, Engelhardt & Ratliff (2019), in a field study in Maryland, found no establishment of buffalograss, blue grama, tufted hairgrass [*Deschampsia caespitosa* (L.) Beauv.], weeping alkaligrass, and prairie dropseed [*Sporobolus heterolepis* (Gray) Gray], but these species may have been limited by non-local and non-roadside adapted germplasm in addition to poor seed germination and high weed pressure.

In general, field studies have found many benefits of turfgrass mixtures over monocultures including more turfgrass coverage (Tyser et al., 1998), less weed coverage (McKernan et al., 2001), less disease incidence over time (Dunn et al., 2002), and improved seasonal color (P. G. Johnson, 2003) if appropriate cool and warm-season species (Bunderson, 2007) and cultivars are mixed (Rimi & Macolino, 2014). When determining appropriate proportions of species in seed mixtures, it is imperative to not incorporate 10% or more by weight of perennial ryegrass or large quantities of other robust early successional species (Dunn et al., 2002), since this will interfere with establishment of long-term species that usually establish more slowly. Balancing the short and long-term benefits of a mixture is important to fulfill the functions that the roadside vegetation was designed to do.

Seed mixtures for roadsides have historically been designed with different levels of diversity. Tüxen (1935) recommended a seed mixture in Germany for the Autobahn that contained up to 15 grass, 4 legume, and 2 herb species; Boeker (1970) later proposed that this mixture was overly complicated and instead recommended simple mixtures from 3-5 species on German roadsides with the addition of low quantities of perennial ryegrass in highly erosive situations. Blaser (1964) recommended 2-4 species and companion grasses if seeding was to occur outside of typical seeding periods for Virginia roadsides. In New Jersey, Duell & Schmit (1975) recommended a simple 1:1:1 ratio of strong creeping red fescue, Chewings fescue or hard fescue, and common-type Kentucky bluegrass, and did not include legume species due to concerns that they would dominate grasses. In France, Henensal et al. (1980) recommended hard fescue, *Festuca pseudovina*, and Chewings fescue, and then later recommended tall fescue in roadside mixtures due to its performance in a drought; the best mixture identified by the authors' consisted of colonial bentgrass [*Agrostis capillaris* L.; syn. *Agrostis tenuis* (Sibth)], hard fescue, and Chewings fescue. In Minnesota, Stenlund & Jacobson (1994) recommended seeding many species for roadside mixtures including both warm and cool-season grasses for uncurbed situations. Biesboer et al. (1998) found that MR-6, the most diverse mixture in the experiment seeded in July, 1993 by Stenlund & Jacobson (1994) consisting of native and nonnative species, was one of the poorest performers in August 1993, 36 months later it was one of the best, although it still only had 36% cover. For central Minnesota curbed roadsides, Friell et al. (2015) recommended a simple mixture of 3 fine fescue taxa.

Mixtures for roadsides and other low-maintenance areas have been recommended with a range in diversity. There is a tendency to only add species to a mixture if there is a visible and measurable benefit (Blaser, 1964); this may be a flawed approach in that it fails to consider the limitations of the study area relative to a region. Therefore, we sought to explore the benefits of seeded turfgrass species richness in a roadside mixture experiment planted at sites throughout Minnesota in two different years. Our objective was to determine if greater turfgrass species richness affects turfgrass, weed, and bare soil coverage. We expected to find a significant benefit of the addition of each additional species over time resulting in more seeded turfgrass coverage, fewer weeds, and less bare soil coverage.

## Materials and Methods

### Research sites

Fourteen research sites were selected across the state of Minnesota (Figure 2.1) to represent a broad range of climatic conditions found in the state. Each research site was immediately adjacent to a curb along a two to four-lane road in full sun conditions, except for Chatfield and Edina, which were partially shaded. Differences in traffic volumes and the amount and type of winter salting were not controlled for. Additionally, there were differences in slope and aspect within and between some sites.

### Species selection

Species selected for this experiment included five cool-season grasses and one warm-season grass (buffalograss) (Table 3.1). The cool-season species were selected based on previous testing and performance in Minnesota (Friell et al., 2012, 2015), and buffalograss was selected based on its adaptability to well-drained, sunny roadsides, since it is an abundant species in the shortgrass prairie, a warmer and drier climate than Minnesota (P. G. Johnson et al., 2001). Cultivars were selected based on their persistence of coverage in a field experiment covering multiple states (Watkins et al., 2019), and/or in a greenhouse experiment assessing the performance of different cultivars to salinity, ice, and heat, which are considered the three most limiting abiotic factors for turfgrass along roadsides (Breuillin-Sessoms & Watkins, 2020; Watkins et al., 2018). Additionally, three check mixtures that are currently seeded along roadsides in Minnesota (25-131, 25-151, MNST-12) (MnDOT, 2014) and one in Michigan (MDOT TUF) were included (Table 3.2).

### Germination testing

To be consistent with the definition of a mixture experiment, the total number of seeds was held constant for each treatment (Cornell, 1973). The total number of pure live seeds (PLS) was determined through germination testing (AOSA, 2016) and purity. Germination for each species was tested with four repetitions of 100 seeds and kept moist with a 2% solution of $KNO_3$. An exception to these rules was that buffalograss germination was defined as the total sum of radicals that emerged from each seed, and germination did not account for potentially dormant or hard seeds. Each plot was seeded at 2 PLS $cm^{-2}$. The weight of each species was determined by counting, weighing, and averaging four repetitions of 1000 seeds. Purity was considered 99% in PLS calculations, unless noted from the seed supplier, except for the four check mixtures for fall 2018 research sites, in this instance the purity was incorrectly specified as 99%.

### Mixture design

Extreme vertices simplex design from the *Xvert* function in the mixexp package in R (R Core Team, 2021) was used to design mixtures (Lawson & Willden, 2016). Buffalograss was limited to 5% total pure live seeds due to its lower seeding rate. The *Fillv* function from the mixexp package was used to add interior points to the mixture design. To reduce the total number of treatments to fit the physical space available we implemented a design optimization algorithm from the *optFederov* function in the AlgDesign package (Wheeler, 2019), and this resulted in an uneven number of treatment combinations with each number of species. This resulted in 36 total seed treatments in addition to the 4 check mixtures that are shown in Table 3.3.

### Experimental design

Forty treatments were seeded at seven sites in each of fall 2018 and fall 2019. Each site had three blocks randomized in a complete block design for a total of 120 plots. Individual plots were 2.3 $m^2$ and adjacent to the curb and perpendicular stretching 1.5 m from the curb. At some sites, there were obstructions such as road signs, hydrants, driveways, and walk-ways and so sections of 1.5-10 m spaces sometimes existed between plots, otherwise there was no buffer between plots. At sites seeded in 2018, 'Navigator II' strong creeping red fescue was seeded in buffer areas and the border behind the plot area. The border behind the plot area was parallel to the road a width of 0-1 m, as space allowed, except for Grand Rapids, which was not seeded in the border area. For research sites seeded in 2019, 'SeaMist' slender creeping red fescue was seeded in these areas.

### Soil sampling and testing

Prior to site establishment, 45-60 soil cores per site were collected by using a small soil core and zig-zagging the plot width in August and September. Cores were sampled to a depth of 10-15 cm at each research site and composited by block. These were analyzed for available phosphorus using the Bray and/or Olsen method, K, organic matter (OM) content, pH, saturated paste extract electrical conductivity, soil texture, and four heavy metals (Fe, Mn, Zn, and Cu) since some are associated with greater weed abundances (Bae et al., 2015). The results of the soil test by research site are shown in Table 3.4. Fine and total soil bulk density was tested at each site between June-August 2020 using the excavation approach and determining the volume with the water method (Page-Dumroese et al., 1999). After removing bulk density soil, it was brought back to the laboratory and weighed, and then sieved using a 2 mm screen, and finally oven dried at 105 ºC until final weight was stable (Page-Dumroese et al., 1999). Bulk density results are shown in Table 3.5. Other physical characteristics calculated were the gravimetric and volumetric fragment content, gravimetric water content, and soil porosity. Additionally, potential plant available water was calculated for each block at all research sites (Saxton et al., 1986).

### Research site establishment

Each site was sprayed 1-2 weeks prior to tillage and then immediately before tillage with a 5% solution of glyphosate (Cornerstone Plus) (WinField Solutions LLC, St. Paul, MN) to kill existing vegetation. Sites were then tilled with a rotary tiller to 10-15 cm depth and raked to smooth the surface and remove excess debris. All plots were seeded by hand and then gently raked in two directions if not overly saturated, in which case no raking occurred (this did not appear to affect plot establishment). A Futerra F4 netless blanket (Profile Products LLC., Buffalo Grove, IL) was then laid over plots and adhered to the surface using 10.2 cm length biostaples (Ecoturf Midwest Inc., Elmhurst, IL). There was some movement of seeds between plots in the second block of Chatfield, where the slope is relatively steep compared to other sites and where a nearby natural spring resulted in greater soil moisture for a portion of that block. A single application of fertilizer was applied to each research site after seeding at a rate of 27.2, 21.4, and 49.7 kg ha$^{-1}$ of N-P-K (10-18-22) (EC Grow Prolinks, EC Grow, Eau Claire, WI).

### Irrigation

Research sites were irrigated with a modular drip irrigation system (Watkins et al., 2020) for 15-49 days after seeding. There were four drip lines each spaced 46 cm apart and the spacing was held between drip lines by securing it with sod staples every 6 m. Plots received 0.4-0.6 cm of water twice per day (8:00 and 13:00). The total number of irrigating days varied between sites due to the date of seeding and if a significant freeze was expected. The Brainerd site was not irrigated by the drip system but instead by a water truck, a common practice for establishing roadside turfgrasses. The water truck irrigated Brainerd five instances of approximately 0.8 cm at each application. No irrigation was applied after the removal of the modular drip irrigation system.

### Plot maintenance

Research sites received no chemical weed control for the duration of the study, except for the Worthington site which was sprayed with a broadleaf herbicide in plots 1-34 in one instance by a lawn care company. No fertilizer was applied after the initial starter application. Most sites were mowed and maintained by our research team, but several were mown by respective municipalities. The mode height of cut for all plots was 8.3 cm usually every 14-21 days (Table 3.6). In a few instances, municipalities accidentally mowed plots shorter at a height between 3.8-6.4 cm. Plots were regularly leaf blown to remove excessive dead plant matter and debris off the plot area, and in the spring some soil debris was occasionally raked or removed by hand from the plots.

## Data collection

The total cover of all research sites was quantified twice per year, once in the fall (Sep.-Nov) and once in the spring (Apr.-Jun.) using the quadrat-grid intersection method (Wilson, 2011). The grid contained an area of 1.16 $m^2$ with 30 intersections spaced regularly and data was collected on two areas of each plot, for a total of 60 data points per plot. Each intersection was a cross of clear fishing line. At the first two sampling periods (fall and spring) coverage was classified as either turfgrass, bare soil, or weeds. Additionally, at the first fall sampling period at all sites, and at one instance the following spring at Grand Rapids, a picture was taken with the grid laying over the plot and coverage at intersections were classified at a later date using the image. Debris was occasionally identified at the point of the intersection from the images, and this was not counted as debris, but instead as missing data, so each instance lowered the total number of intersections (or data points) for a particular plot. All subsequent sampling, starting approximately one year after seeding was classified into one of the five species that were seeded (Table 3.1); hard fescue and slender creeping red fescue are difficult to distinguish so were grouped into the same classification of “fine fescue”. Other classifications consisted of perennial ryegrass (which was included in three of the check mixtures), white clover (a common roadside broadleaf at many sites), sedge, rush, other grass, other broadleaf, bare soil, or a tree sapling. The dates that each site was seeded and sampled is shown in Table 3.7.

## Statistics

All analysis and data preparation were conducted using the open-source software R (R Core Team, 2021). A generalized linear mixed model (GLMM) was conducted in the lme4 package (Bates et al., 2014) using the *glmer* function with family set to binomial.

The three primary response variables were seeded turfgrass, weed, and bare soil coverage (%). The fixed effect predictor variables were the season of sampling (fall or spring), time defined as the order of vegetation sampling (i.e. 1=first-time sampling vegetation, 2=second-time sampling vegetation, etc), number of species seeded in the treatment (1-6), and the interaction between time and number of species. Research site was included as a random effect. Model selection was guided by minimizing the AIC score for generalized linear mixed models

Spatial stability of both turfgrass and weed cover was calculated by the mean divided by the standard deviation (Lehman & Tilman, 2000). The average coverage and standard deviation were calculated from all research sites and blocks composited resulting in a total of 360 observations (40 treatments x 5 sampling times for sites seeded in 2018 + 40 treatments x 4 sampling times for sites seeded in 2019). The subsequent dataset contains average coverage and standard deviation for 40 treatments (Table 3.3) at different sampling times for sites seeded in either 2018 or 2019.

Two linear models using the *lm* function in R were developed with the spatial stability of turfgrass and weed cover as response variables. The response variables were natural log transformed based on the results of a box-cox analysis. A small value of 0.1 was added to weed spatial stability prior to transformation to reduce undefined values; this was not an issue for turfgrass spatial stability values. Linear model estimates are shown exponentiated to simplify model interpretation. The predictor variables included in the model were the seeding year (2018 or 2019), season of sampling (fall or spring), time (1-5), and number of species (1-6). The linear model selection was guided by maximizing the $R^2$. An effort was made in all model selection to reduce complexity and only include the most relevant main and interaction effects. Statistical assumptions were analyzed graphically, and some minor deviations were present on the lower and upper portions of the normality of error assumption on the linear models. All results containing the number of species relate to the number of seeded species within a treatment and not necessarily the number of observed species.

## Results

### Turfgrass coverage

Differences in turfgrass coverage by site and number of species seeded in each treatment are shown in Figure 3.1. The GLMM analysis for turfgrass coverage showed a significant positive interaction between time, defined as the order of sampling instances, and number of species (Est=0.08, S.E.=0.02, $P<0.001$) (Table 3.8). The predicted effects of the interaction between time and number of species are shown in Figure 3.2 with the effect of additional species resulting in greater turfgrass coverage as time increases. Average turfgrass coverage, standard deviation, and spatial stability for each treatment is shown in Fig. 3.3-4. A linear model showed that a one-unit change in the number of species resulted in 1.05-1.12 times increase in turfgrass spatial stability. ($F_{4,355}=8.34$, $P<0.001$) (Table 3.9). Each additional time increment was found to result in 1.00-1.07 times increase in turfgrass spatial stability ($P=0.04$) (Table 3.9).

### Weed coverage

No significant interaction effect existed for number of species and time on weed cover in the GLMM analysis ($P=0.08$) (Table 3.8). When the time by number of species interaction effect was not included in the model for weed coverage, the main effect of the number of species was highly significant (Est=-0.55, S.E.=0.05, $P<0.001$) and negatively associated with weed coverage. When sampling total coverage in the spring, there was significantly less weed coverage (Est=-1.03, $P<0.001$). A one-unit increase in time resulted in significantly more logged odds of weed coverage (Est=0.88, $P<0.001$). Average weed coverage, standard deviation, and spatial stability for each treatment is shown in Fig. 3.5-6. Weed spatial stability resulted in 0.92-1.00 times decrease ($F_{4,355}=79.8$, $P=0.03$) with the increase of each additional turfgrass species (Table 3.10). Each additional time increment was found to result in 1.34-1.44 times increase in weed spatial stability ($P<0.001$) (Table 3.10).

### Bare soil coverage

No significant interaction effect existed between number of species and time affecting bare soil coverage in the GLMM analysis (P=0.37) (Table 3.8), although there was a significant effect of the increase in each additional turfgrass species resulting in lower coverage of bare ground (Est=-0.14, P=0.02). When sampling total coverage in the spring, there was significantly more bare soil coverage (Est=0.67, P<0.001). A one-unit increase in time resulted in significantly less bare soil coverage (Est=-1.14, P<0.001) (Table 3.8).

## Discussion

The importance of roadside turfgrass species diversity for maintaining persistent cover has been understated previously. We found greater turfgrass species richness increased turfgrass coverage over time, resulted in less weed coverage, and less bare soil. Additionally, more species in a mixture had greater turfgrass spatial stability. These findings support the development of roadside seed mixtures containing more species for transportation agencies. In some studies, there has been a tendency to simplify roadside mixtures by including few species (Blaser, 1964; Boeker, 1970; Friell et al., 2015), but many of these experiments were conducted at one or two research sites, and so results would likely show only a few top performers. Seed mixtures have been simplified for different planting environments such as by drainage classes (Boeker, 1970), but this should not reduce mixture diversity to only a few species, even if drainage class and proximity to the road are similar, since this experiment showed there are benefits of increasing species richness.

Our study shows that the benefits of greater cover of seeded species diversity in natural environments can extend to managed roadside landscapes. Non-roadside ecological studies have shown that greater diversity can improve field biomass and other ecosystem functioning (Mueller et al., 2013). The insurance effect hypothesis proposes that including more species results in some that are better adapted to adverse conditions than others, effectively maintaining productivity and multifunctionality (Yachi & Loreau, 1999) and this has been verified in field studies (Minns et al., 2001; Tilman & Downing, 1994). Greater species diversity can also act as spatial insurance (Loreau et al., 2003). Furthermore, the insurance effect stresses the importance of greater species asynchrony, since that results in a decrease in the variance of coverage (Yachi & Loreau, 1999). Previous testing along roadsides has found different performance of individual species and cultivars (Friell et al., 2012; Friell & Watkins, 2020), and these differences are likely based on climate, edaphic conditions of a site, and disturbances. Species asynchrony and response diversity (Sasaki et al., 2019) of adapted species and cultivars to roadsides should be included in the design of roadside mixtures; this can be achieved by including more diversity at the species level, and then including additional cultivars within a species (Barot et al., 2017).

A question arises as to whether sowing complete plant communities should be the primary goal for roadsides revegetation efforts or rather seeding simpler mixtures that later allow for the colonization of other beneficial species (Boeker, 1970; Soper et al., 2019b). One concern with this approach is that it could encourage other non-desirable species from establishing at these sites. Surveys of roadside vegetation can provide insights into what species are persisting at these harsh sites, along with their relative abundances. Based on a roadside vegetation survey in western Germany, Tüxen & Lohmeyer (1961) found relatively few species dominated roadsides and therefore they recommended four mixtures each containing 6 grass species. When roadside vegetation was surveyed in Poland, Żołnierczuk & Fornal-Pieniak (2020) found strong creeping red fescue ranged from 30-50% cover with the *Poaceae* family cumulatively accounting for 80% of the coverage along roadsides. They additionally found the *Asteraceae* and *Fabaceae* plant families to be increasing on older roads, along with approximately 20% annual species, and so they recommended the inclusion of adapted annual, *Asteraceae*, and *Fabaceae* species in roadside seed mixtures. Other roadside vegetation surveys have found a considerable amount of plant biodiversity on roadsides illustrating that they can harbor rare and important plant biodiversity (Arenas et al., 2017; Brown & Sawyer, 2012). This biodiversity can have significant variability across space but composition can be relatively similar within a single state (Rentch et al., 2013). Surveys in Minnesota from 1-3 m next to the road showed that in the majority of instances there are fewer species closer to the curb than in areas further from the curb, and bare ground coverage decreased with distance from the curb (Biesboer et al., 1998). White & Smithberg (1972) found that when seeding mixtures of different turfgrass species that some dominated the moister sections of the boulevard and others the drier portions. These vegetation surveys collectively indicate that many species are likely to exist or find a niche on roadsides and so invasion of both beneficial and weedy species is likely.

Our findings show that seeding greater species richness will allow greater turfgrass cover, but it is important to design mixtures with appropriate proportions, otherwise the benefits of greater richness would be reduced. We observed that tall fescue and slender creeping red fescue, two of the quickest establishing species included in our study, can reduce the establishment of hard fescue, Kentucky bluegrass, and buffalograss, which are all slower establishing species. On roadsides in Minnesota, Friell et al. (2015) found that the coverage of tall fescue was found to be lower than the original proportion in its seed mixture, so reducing the proportion of this species in mixtures may not only allow for better establishment of other species, but be more cost-efficient. When hard fescue, a slower establishing species, was a top performer at a site, we found that its monoculture performance was sometimes better than the high diversity mixtures. The long-term advantage of hard fescue has been noted before on roadsides in Minnesota (Friell et al., 2012), and it has been underutilized in historical roadside turfgrass mixtures recommended for the state (MnDOT, 2014).

There remains a tension between the proportion of short and persistent long-term coverage when designing a roadside mixture. In general, to allow for the maximum benefits of a high species richness mixture, it is important to limit the proportion of quickly establishing species. A mixture with many species will have improved community stability, but each individual will have reduced stability (Lehman & Tilman, 2000), and so balancing the establishment of all species over a few could improve spatial and temporal stability. Previous research in both roadside and non-roadside settings have found that including perennial ryegrass greater than or equal to 10% by weight can reduce the quality of other species in a mixture, since perennial ryegrass establishes very quickly (Dunn et al., 2002; Henensal et al., 1980).

Establishing and maintaining vegetation along roadsides is difficult and seed mixtures have historically been designed with varying levels of diversity, but often with too little diversity. Roadsides also contain differences in environmental factors such as the climate, soil physical and chemical characteristics, disturbance, and management. Our findings show that when planting across numerous research sites there is a measurable increase in turfgrass coverage with the addition of each species over time. A potential limitation of this study is that data collection for sites seeded in 2018 occurred for two-years, and one and a half years for plots seeded in 2019, and we know the abundance of some species are more rapidly changing at some sites. Overall, we recommend including greater species in seed mixtures for roadsides to provide more coverage that is also more spatially stable. This will result in roadside vegetation that continues to reduce soil erosion, provides a short-stature and aesthetically uniform landscape, and maintains safe pathways for drivers.

Table 3.1: Species and cultivars chosen for this experiment. This list does not include additional species and cultivars that were included with check mixtures.

| Common name | Scientific name | Cultivar |
|---|---|---|
| Buffalograss | *Buchloe dactyloides* (Nutt.) Engelm. | Sundancer |
| Hard fescue | *Festuca brevipila* Tracey | Gladiator |
| Kentucky bluegrass | *Poa pratensis* L. | Tirem |
| Slender creeping red fescue | *Festuca rubra* L. ssp. *littoralis* (G. Mey.) Auquier | SeaMist |
| Tall fescue | *Schedonorus arundinaceus* (Schreb.) Dumort. | Saltillo |
| Weeping alkaligrass | *Puccinellia distans* (Jacq.) Parl. | Sea Salt |

Table 3.2: Species components of seed mixtures for roadsides that were included in the study. The Michigan check mixture is MDOT TUF while other mixtures are recommended by MnDOT.

| DOT check mixture name[a] | Seed lot number | Species scientific name | Species common name | Variety | Weight (%)[b] | Approx. seed ratio of mixture (%)[c] |
|---|---|---|---|---|---|---|
| MDOT TUF | L152-18-459 | *Festuca rubra* L. ssp. *rubra* Gaudin | Strong creeping red fescue | Epic | 38.32 | 32.05 |
| | | *Festuca brevipila* Tracey | Hard fescue | Reliant IV | 19.91 | 16.03 |
| | | *Lolium perenne* L. | Perennial ryegrass | Palmer III | 19.02 | 5.95 |
| | | *Puccinellia distans* (Jacq.) Parl. | Weeping alkaligrass | Salty | 9.99 | 16.30 |
| | | *Poa pratensis* L. | Kentucky bluegrass | Arc | 9.92 | 29.68 |
| 25-131 | 18225A | *Festuca rubra* L. ssp. *rubra* Gaudin | Strong creeping red fescue | Boreal | 29.09 | 21.79 |
| | | *Festuca rubra* L. ssp. *commutata* Gaudin | Chewings fescue | Fairmont | 20.00 | 12.18 |
| | | *Poa pratensis* L. | Kentucky bluegrass | Blue Angel | 16.36 | 43.83 |
| | | *Festuca brevipila* Tracey | Hard fescue | Jetty | 13.64 | 9.83 |
| | | *Festuca ovina* L. | Sheep fescue | Blue Ray | 11.37 | 9.69 |
| | | *Lolium perenne* L. | Perennial ryegrass | Royal Green | 9.54 | 2.67 |
| 25-151 | 18218A | *Poa pratensis* L. | Kentucky bluegrass | Blue Angel | 25.00 | 31.64 |
| | | *Poa pratensis* L. | Kentucky bluegrass | Park | 25.00 | 31.64 |
| | | *Poa pratensis* L. | Kentucky bluegrass | Merit | 25.00 | 31.64 |
| | | *Lolium perenne* L. | Perennial ryegrass | Shining Star | 17.00 | 2.25 |
| | | *Festuca rubra* L. ssp. *rubra* Gaudin | Strong creeping red fescue | Boreal | 8.00 | 2.83 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MNST-12 (2018) | 18238B | *Festuca rubra* L. ssp. *rubra* Gaudin | Strong creeping red fescue | Cardinal | 19.91 | 13.78 |
| | | *Festuca rubra* L. ssp. *commutata* Gaudin | Chewings fescue | Radar | 19.62 | 11.04 |
| | | *Festuca brevipila* Tracey | Hard fescue | Jetty | 19.75 | 13.16 |
| | | *Poa pratensis* L. | Kentucky bluegrass | Blue Note | 19.60 | 48.53 |
| | | *Festuca rubra* L. ssp. *littoralis* (G. Mey.) Auquier | Slender creeping red fescue | Seabreeze GT | 19.49 | 13.49 |
| MNST-12 (2019) | 19142B | *Festuca rubra* L. ssp. *commutata* Gaudin | Chewings fescue | Radar | 19.96 | 11.09 |
| | | *Festuca rubra* L. ssp. *littoralis* (G. Mey.) Auquier | Slender creeping red fescue | Shoreline | 19.95 | 13.64 |
| | | *Poa pratensis* L. | Kentucky bluegrass | Diva | 19.94 | 48.76 |
| | | *Festuca brevipila* Tracey | Hard fescue | Beacon | 19.93 | 13.11 |
| | | *Festuca rubra* L. ssp. *commutata* Gaudin | Strong creeping red fescue | Epic | 19.61 | 13.40 |

[a] Different MNST-12 seed lots were used in different planting years incorporating similar species ratios but only similarity in a single cultivar (‘Radar’).

[b] Proportion of seed weight in each mixture.

[c] Estimated proportion of the number of seeds in each mixture based on number of seeds per weight.

Table 3.3: The proportion of pure live seed ratios for each species in each of the 40 treatments. MDOT TUF is the Michigan check mixture. 25-131, 25-151, and MNST-12 are recommended by MnDOT.

| Treatment | Buffalograss | Tall fescue | Slender creeping red fescue | Kentucky bluegrass | Weeping alkaligrass | Hard fescue |
|---|---|---|---|---|---|---|
| 1 | 100 | | | | | |
| 2 | | 100 | | | | |
| 3 | | | 100 | | | |
| 4 | | | | 100 | | |
| 5 | | | | | 100 | |
| 6 | | | | | | 100 |
| 7 | 5 | 95 | | | | |
| 8 | 5 | | 95 | | | |
| 9 | 5 | | | 95 | | |
| 10 | 5 | | | | 95 | |
| 11 | 5 | | | | | 95 |
| 12 | | | | 50 | | 50 |
| 13 | | | | 50 | 50 | |
| 14 | | | 50 | | | 50 |
| 15 | | | 50 | | 50 | |
| 16 | | | 50 | 50 | | |
| 17 | | 50 | | | | 50 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 18 | | 50 | | | 50 | |
| 19 | | 50 | | 50 | | |
| 20 | | 50 | 50 | | | |
| 21 | 2.5 | | | | 97.5 | |
| 22 | 5 | | | | 47.5 | 47.5 |
| 23 | 5 | | | 47.5 | | 47.5 |
| 24 | 5 | | | 47.5 | 47.5 | |
| 25 | 5 | | 47.5 | | | 47.5 |
| 26 | 5 | | 47.5 | | 47.5 | |
| 27 | 2.5 | 97.5 | | | | |
| 28 | 5 | 47.5 | | | | 47.5 |
| 29 | 5 | 47.5 | | | 47.5 | |
| 30 | 5 | 47.5 | | 47.5 | | |
| 31 | 5 | 47.5 | 47.5 | | | |
| 32 | 2.5 | | 48.75 | 48.75 | | |
| 33 | | | | | 50 | 50 |
| 34 | 2.5 | 47.5 | | | | 50 |
| 35 | 2.5 | | | | 47.5 | 50 |
| 36 | 2.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 |
| 37 | MNST-12 | | | | | |
| 38 | 25-131 | | | | | |
| 39 | 25-151 | | | | | |

| | |
|---|---|
| 40 | MDOT TUF |

Table 3.4: Average soil chemical properties for each research site. Greater differences exist between than within a site. Bray P and Olsen P is the available phosphorus in the soil. Bray is reliable when the pH is less than 7.4 and Olsen when it is greater than 7.4. Soil testing was analyzed by the University of Minnesota soil testing laboratory using standard methods.

| Research site | Bray P[b] | Olsen P[b] | K[b] | OM | pH | Sat. elect. conductivity (mmhos/cm)[a] | Sand (%) | Silt (%) | Clay (%) | Soil texture[c] | Fe[ab] | Mn [ab] | Zn [ab] | Cu [ab] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bemidji | 38.33 | NA | 82.00 | 2.07 | 7.30 | 1.07 | 69.57 | 10.42 | 20.01 | SCL | 19.20 | 5.44 | 2.84 | 0.34 |
| Brainerd | 28.67 | 9.00 | 78.33 | 3.10 | 7.40 | 0.73 | 71.23 | 7.07 | 21.67 | SCL | 58.46 | 4.67 | 43.29 | 14.35 |
| Chatfield | 25.67 | 12.00 | 133.00 | 2.70 | 7.50 | 0.77 | 52.50 | 15.87 | 31.70 | SCL | 22.99 | 8.17 | 1.43 | 0.38 |
| Duluth | 10.00 | 4.67 | 56.00 | 2.60 | 7.50 | 1.63 | 55.01 | 22.91 | 22.07 | SCL | 57.07 | 6.22 | 3.74 | 4.70 |
| E. G. Forks | NA | 10.33 | 239.67 | 4.97 | 7.87 | 0.63 | 3.93 | 48.60 | 47.53 | Silty clay | 10.14 | 6.51 | 1.71 | 1.40 |
| Edina | 26.00 | NA | 69.67 | 5.80 | 7.20 | 1.43 | 49.20 | 25.40 | 25.40 | SCL | 40.99 | 4.69 | 6.74 | 1.11 |
| Fergus Falls | 15.67 | 7.33 | 230.67 | 5.10 | 7.90 | 0.97 | 50.40 | 19.63 | 30.03 | SCL | 13.09 | 4.54 | 6.17 | 1.15 |
| G. Rapids | 38.33 | 10.67 | 59.33 | 1.83 | 7.43 | 0.80 | 60.00 | 18.33 | 21.70 | SCL | 43.00 | 7.12 | 2.95 | 1.03 |
| Int. Falls | 4.67 | 4.00 | 129.33 | 6.63 | 7.53 | 1.10 | 41.20 | 22.10 | 36.70 | Clay loam | 40.00 | 2.03 | 3.49 | 1.19 |
| Marshall | 8.67 | 11.00 | 203.33 | 4.00 | 7.77 | 0.47 | 34.40 | 23.50 | 42.07 | Clay | 20.25 | 7.62 | 3.41 | 1.19 |
| Roseville | 14.50 | 6.17 | 74.33 | 5.13 | 7.88 | 0.63 | 61.67 | 13.37 | 25.00 | SCL | 31.38 | 3.89 | 12.82 | 2.59 |
| Saint Cloud | 46.67 | NA | 142.33 | 2.70 | 6.87 | 1.40 | 58.31 | 15.42 | 26.26 | SCL | 64.36 | 8.37 | 2.42 | 0.53 |
| Willmar | 3.00 | NA | 183.00 | 3.43 | 7.47 | 1.53 | 42.49 | 26.26 | 31.26 | Clay loam | 17.73 | 4.11 | 5.97 | 1.02 |
| Worthington | 21.00 | NA | 170.00 | 5.03 | 7.30 | 0.83 | 16.26 | 39.58 | 44.16 | Clay | 29.06 | 7.51 | 2.88 | 1.01 |

[a] Saturated paste extract electrical conductivity, Fe, Mn, Zn, and Cu analysis from sites seeded in the fall of 2018 came from additional soil samples collected in summer 2020.

[b] Units of mg kg$^{-1}$.

[c] SCL = sandy clay loam.

Table 3.5: Fine and total bulk density from each zone (Curb, Mid, Far) within a research site.

| | Bulk density (g $cm^{-3}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Fine | | | | Total (Coarse) | | | |
| Research site | Curb[a] | Mid[b] | Far[c] | Avg. | Curb[a] | Mid[b] | Far[c] | Avg. |
| Bemidji | 1.36 | 1.39 | 1.39 | 1.38 | 1.41 | 1.43 | 1.47 | 1.43 |
| Brainerd | 1.47 | 1.27 | 1.29 | 1.34 | 1.51 | 1.35 | 1.34 | 1.40 |
| Chatfield | 1.24 | 1.19 | 1.23 | 1.22 | 1.29 | 1.24 | 1.30 | 1.28 |
| Duluth | 1.28 | 1.22 | 1.44 | 1.31 | 1.64 | 1.44 | 1.74 | 1.61 |
| E. G. Forks | 1.09 | 1.11 | 1.14 | 1.11 | 1.13 | 1.13 | 1.16 | 1.14 |
| Edina | 1.42 | 1.17 | 1.26 | 1.28 | 1.50 | 1.22 | 1.33 | 1.35 |
| Fergus Falls | 1.20 | 1.20 | 1.16 | 1.19 | 1.29 | 1.31 | 1.23 | 1.28 |
| Grand Rapids | 1.49 | 1.56 | 1.70 | 1.58 | 1.62 | 1.71 | 1.81 | 1.71 |
| Int. Falls | 1.07 | 0.82 | 0.96 | 0.95 | 1.18 | 0.92 | 1.05 | 1.05 |
| Marshall | 1.13 | 1.08 | 1.08 | 1.10 | 1.18 | 1.17 | 1.19 | 1.18 |
| Roseville | 1.29 | 1.18 | 1.21 | 1.23 | 1.32 | 1.23 | 1.27 | 1.27 |
| Saint Cloud | 1.30 | 1.37 | 1.41 | 1.36 | 1.38 | 1.42 | 1.48 | 1.43 |
| Willmar | 1.26 | 1.23 | 1.38 | 1.29 | 1.32 | 1.31 | 1.46 | 1.36 |
| Worthington | 1.01 | 1.08 | 1.06 | 1.05 | 1.06 | 1.16 | 1.11 | 1.11 |
| Average | 1.26 | 1.21 | 1.26 | 1.24 | 1.35 | 1.29 | 1.35 | 1.33 |

[a] Core sampled immediately adjacent to the curb.

[b] Core sampled 0.8 m away from the curb in the center of the plot.

[c] Core sampled 1.5 m away from the curb on the inside edge of the plot.

Table 3.6: Mowing and other maintenance details at each research sites. We found it difficult to control mowing height and frequency even with preventative measures at some sites.

| Research site | Growing year | Mode mowing height (in) | Mode mowing height (cm) | Total number of mows | Average mowing interval (days) | Comments related to mowing and other maintenance |
|---|---|---|---|---|---|---|
| Bemidji | 2020 | 3.25 | 8.3 | 6 | 22.6 | |
| Brainerd | 2019 | 3.25 | 8.3 | 7 | 18.3 | |
| Brainerd | 2020 | 3.25 | 8.3 | 9 | 17.0 | |
| Chatfield | 2019 | 3.25 | 8.3 | 6 | 29.2 | Plots were mown infrequently in both growing years due to little aboveground growth from drought conditions. |
| Chatfield | 2020 | 3.25 | 8.3 | 7 | 29.3 | |
| Duluth | 2020 | 3.25 | 8.3 | 5 | 23.5 | |
| E. G. Forks | 2019 | 3 | 7.6 | 6 | 20.6 | |
| E. G. Forks | 2020 | 3 | 7.6 | 10 | 12.0 | Municipality mowed the plots every 10-14 days at 7.6 cm. Their heavy mowers resulted in some dead grass from the wheel traffic in the center of the plots. |
| Edina | 2020 | 3.25 | 8.3 | 11 | 15.1 | Occasionally the border to the last portion of the plot furthest from the curb was mown by us at 9.5 cm to avoid scalping the grass in the plot due to the change in contour. After mowing ceased by us, the municipality began mowing this section of the plots close to 5 cm. |
| Fergus Falls | 2019 | 3.25 | 8.3 | 7 | 19.5 | |
| Fergus Falls | 2020 | 3.25 | 8.3 | 9 | 16.8 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Grand Rapids | 2019 | 3.25 | 8.3 | 6 | 25.4 | Grand Rapids municipality mowed plots three times total over the period of data collection (2019-2020). Each time they mowed it around 3.8-5.1 cm to the detriment of the site. |
| Grand Rapids | 2020 | 3.25 | 8.3 | 8 | 17.9 | After we finished all data collection, we observed the municipality was mowing the boulevard at 5.1 cm which hindered performance of all grasses disproportionately at this site. |
| International Falls | 2020 | 3 | 7.6 | 10 | 7.0 | Blocks 1-2 were mown by local municipality at a height of 5.1-6.4 cm and block 3 was mown by a homeowner at 7.6 cm. |
| Marshall | 2019 | 2 | 5.1 | 14 | 7.0 | The nearby golf course mowed this research site approximately every 7 days at 5 cm or shorter occasionally. There were some periods where they left it a little taller and mowed it with a push mower. |
| Marshall | 2020 | 2 | 5.1 | 14 | 7.0 | |
| Roseville | 2019 | 3.25 | 8.3 | 7 | 18.7 | This site was mowed once at 5.6 cm in May 2019. |
| Roseville | 2020 | 3.25 | 8.3 | 9 | 19.1 | |
| Saint Cloud | 2020 | 3.25 | 8.3 | 8 | 16.1 | Starting around 07/23/20, or likely sooner, plots 75-120 were mowed around 3.8-5.1 cm. Block 3 was found to have less turfgrass coverage and more crabgrass coverage, likely in part due to poor mowing practices, but also potentially due to lower organic matter content in this block. |
| Willmar | 2020 | 3.25 | 8.3 | 8 | 16.1 | |
| Worthington | 2020 | 3.25 | 8.3 | 9 | 23.6 | Plots 1-34 were mowed and then sprayed, most likely with a broadleaf herbicide by a lawn care company several days prior to 05/21/20. On a different occasion, several days prior to 11/05/20, plots ~30-60 3 ft from the curb to the sidewalk (1.5 m) were mowed by a resident at 5.1 cm. |

Table 3.7: The date of seeding and total coverage sampling for all research sites. An NA occurs when sampling did not take place.

| Research site | Seeding date | Fall 2018 | Spring 2019 | Fall 2019 | Spring 2020 | Fall 2020 | Spring 2021 |
|---|---|---|---|---|---|---|---|
| G. Rapids | 8/30/18 | 10/19/18 | 5/31/19 | 10/11/19 | 5/28/20 | 9/21/20 | NA |
| Brainerd | 9/12/18 | 10/19/18 | 5/28/19 | 10/2/19 | 5/26/20 | 10/9/20 | NA |
| E. G. Forks | 9/13/18 | 10/18/18 | 5/29/19 | 10/10/19 | 6/2/20 | 10/2/20 | NA |
| Fergus Falls | 9/14/18 | 10/18/18 | 5/23/19 | 10/9/19 | 5/20/20 | 10/1/20 | NA |
| Roseville | 9/15/18 | 10/26/18 | 5/20/19 | 9/27/19 | 5/18/20 | 10/19/20 | NA |
| Marshall | 9/17/18 | 10/25/18 | 5/15/19 | 11/1/19 | 5/6/20 | 11/6/20 | NA |
| Chatfield | 9/18/18 | 11/1/18 | 5/10/19 | 10/30/19 | 5/7/20 | 10/30/20 | NA |
| Bemidji | 8/26/19 | NA | NA | 10/16/19 | 5/27/20 | 9/18/20 | 5/21/21 |
| Int. Falls | 8/28/19 | NA | NA | 10/17/19 | 6/3/20 | 9/14/20 | 5/26/21 |
| Duluth | 9/6/19 | NA | NA | 10/18/19 | 6/5/20 | 9/25/20 | 5/25/21 |
| Saint Cloud | 9/18/19 | NA | NA | 10/23/19 | 5/19/20 | 10/14/20 | 5/14/21 |
| Willmar | 9/11/19 | NA | NA | 10/22/19 | 5/15/20 | 10/16/20 | 4/30/21 |
| Edina | 8/30/19 | NA | NA | 10/15/19 | 5/14/20 | 10/13/20 | 4/23/21 |
| Worthington | 9/4/19 | NA | NA | 10/25/19 | 4/30/20 | 11/5/20 | 4/16/21 |

Table 3.8: Generalized linear mixed effects model (GLMM) summary output with three primary response variables of turfgrass, weed, and bare soil coverage. All response variables are a proportion untransformed and bounded from 0-1. Research site was included as a random effect. The number of experimental sampling units is 7,560 for this analysis (N=7,560). Time is the order of sampling instances. Research sites seeded in 2018 were sampled 5 times and sites seeded in 2019 were sampled 4 times.

| | Turfgrass coverage | | | Weed coverage | | | Bare soil coverage | | |
|---|---|---|---|---|---|---|---|---|---|
| | Estimate | Std. error | Pr(>\|z\|) | Estimate | Std. error | Pr(>\|z\|) | Estimate | Std. error | Pr(>\|z\|) |
| (Intercept) | -0.78929 | 0.34404 | 0.02180 | -3.71137 | 0.36300 | <2e-16 | 1.55864 | 0.25861 | 1.67E-09 |
| Season spring | 0.24500 | 0.05550 | 1.01E-05 | -1.02851 | 0.09944 | <2e-16 | 0.66581 | 0.07069 | <2e-16 |
| Time | 0.07398 | 0.04915 | 0.13230 | 0.88029 | 0.09461 | <2e-16 | -1.13551 | 0.07781 | <2e-16 |
| Number of species | 0.09311 | 0.05351 | 0.08190 | -0.28536 | 0.15500 | 0.06560 | -0.14086 | 0.06258 | 0.02440 |
| Time:Number of species | 0.08161 | 0.01841 | 9.33E-06 | -0.07169 | 0.04040 | 0.07600 | -0.02639 | 0.02950 | 0.37100 |

Figure 3.1: The average turfgrass coverage based on the number of turfgrass species when sampling occurred in fall 2020 across all 14 research sites.

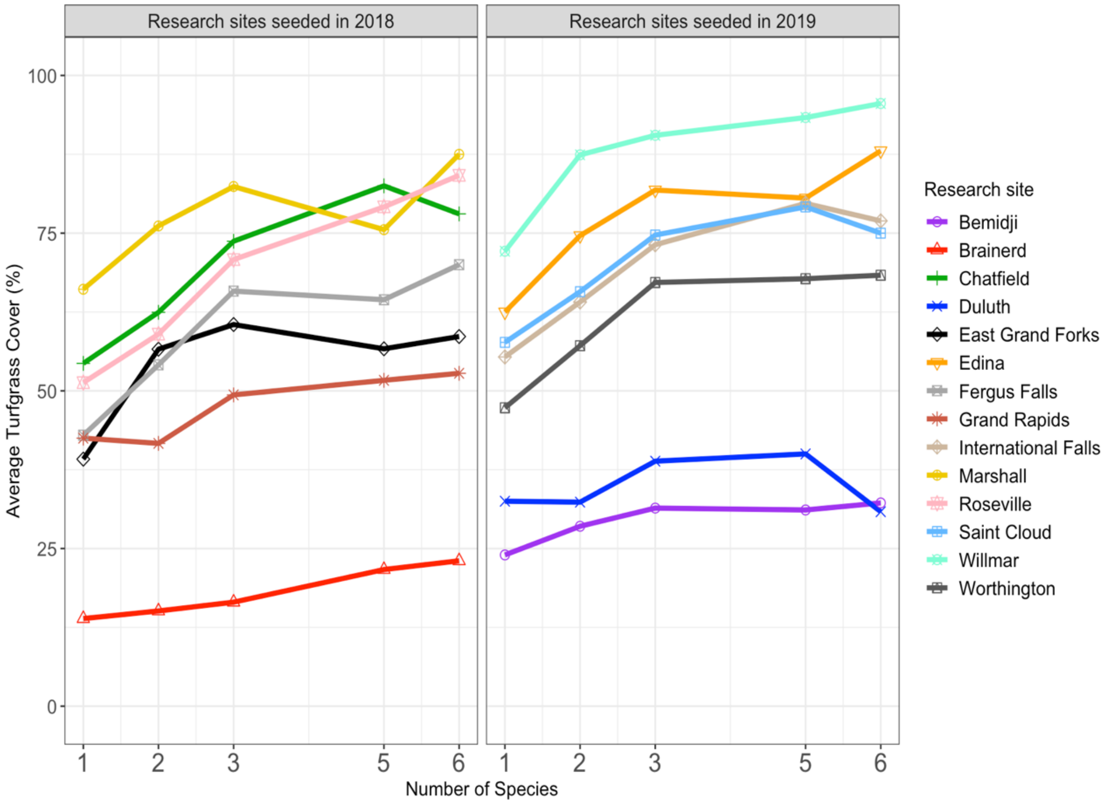

Figure 3.2: GLMM predictions of the interaction effect between time and the number of species for the plot area covered by turfgrass, weed, or bare soil. Odd time increments were sampled in the fall and even in the spring. Error bars show the 95% confidence interval. N. Spp. is the number of species included in the seed treatment at the time of seeding. The fifth sampling time contains data only from sites seeded in 2018.

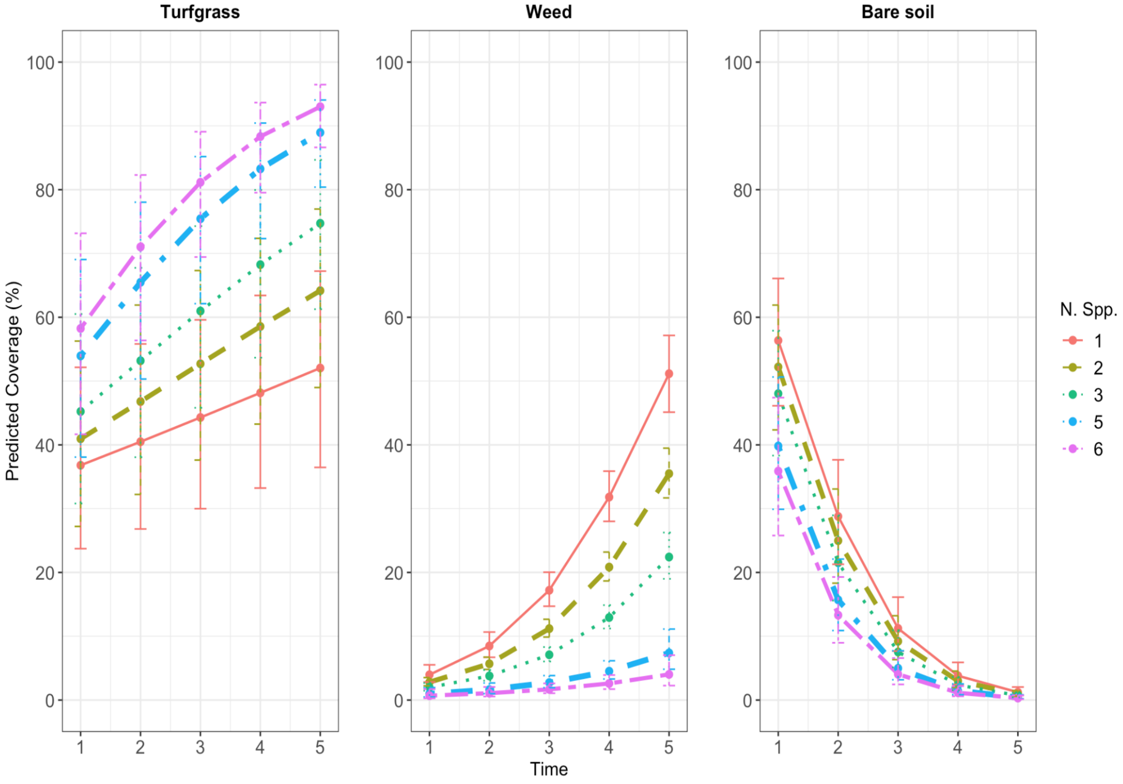

Figure 3.3: Mean, standard deviation, and spatial stability (mean/standard deviation) of turfgrass coverage for each seed treatment for sites seeded in 2018. The addition of alkaligrass in a seed mixture resulted in significantly lower standard deviation in spring 2019, since that was the only species that performed adequately at one site (see open space in the center of the plot). St. dev = standard deviation. Vegetation sampling season and year are shown facetted in the columns.

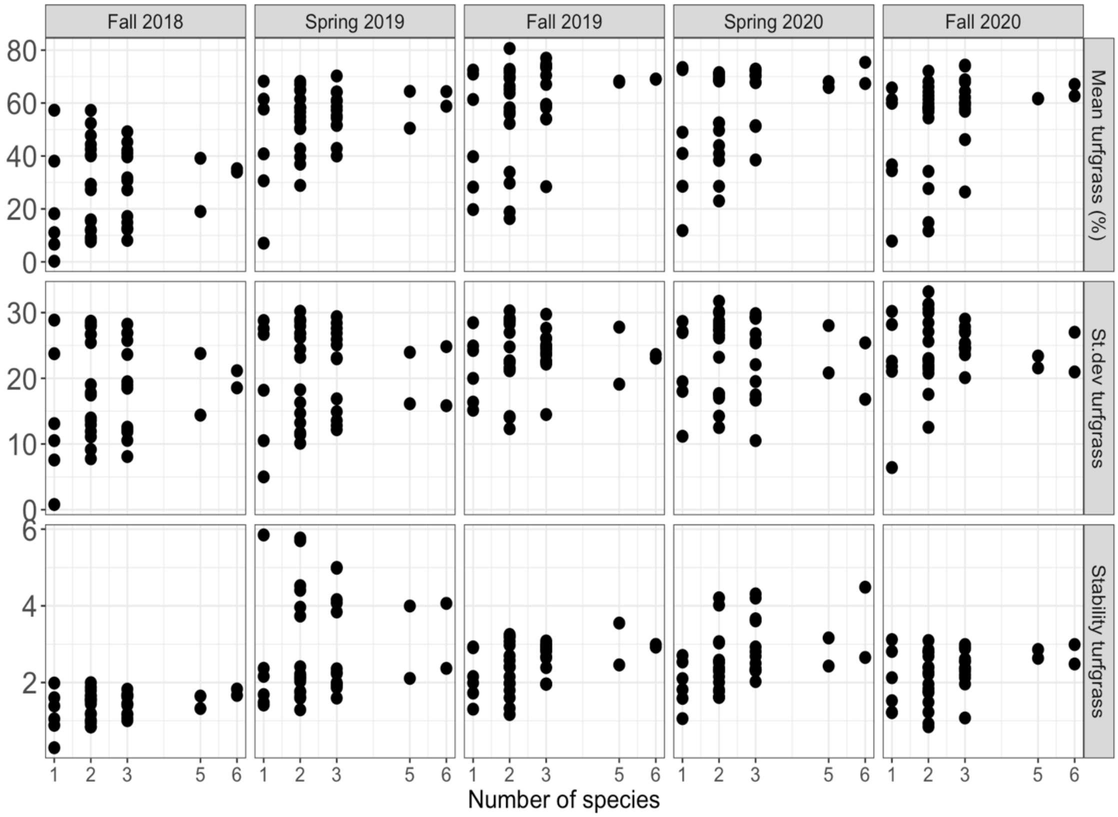

Figure 3.4: Mean, standard deviation, and spatial stability (mean/standard deviation) of turfgrass coverage for each seed treatment for sites seeded in 2019. St. dev = standard deviation. Vegetation sampling season and year are shown facetted in the columns.

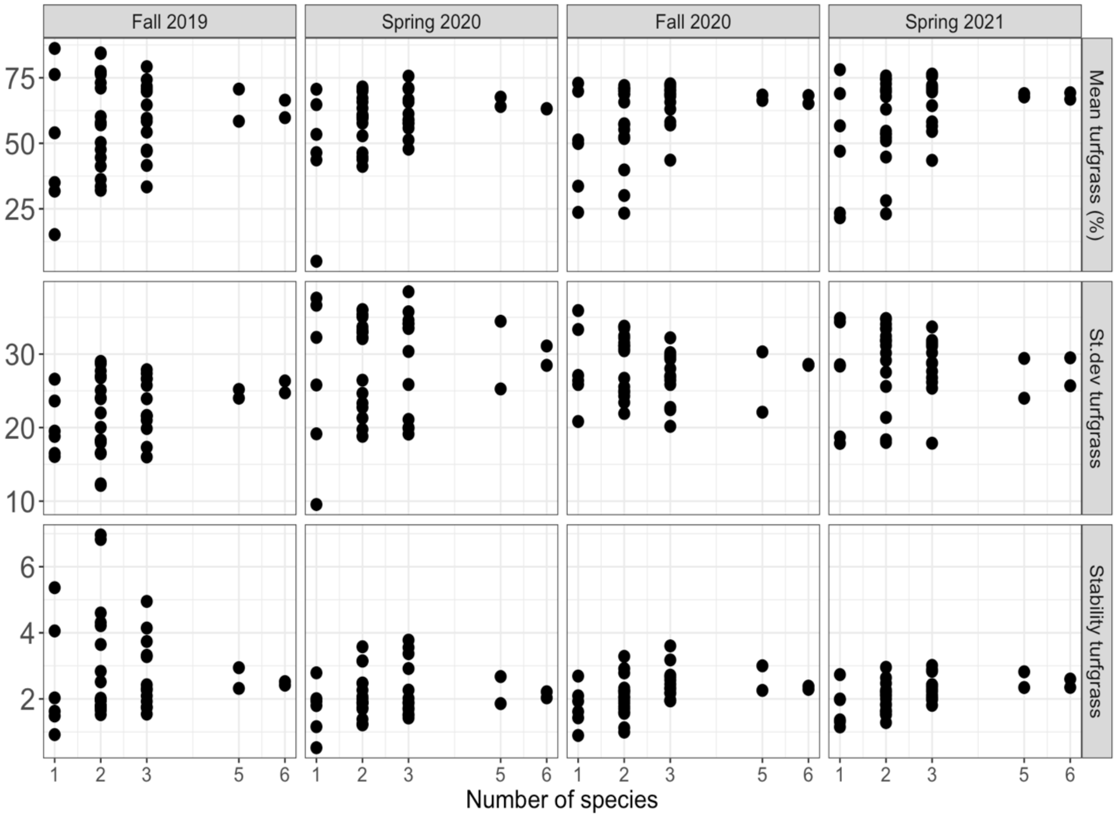

Figure 3.5: Mean, standard deviation, and spatial stability (mean/standard deviation) of weed coverage for each seed treatment for sites seeded in 2018. St. dev = standard deviation. Vegetation sampling season and year are shown facetted in the columns. Undefined stability values changed to 0.1.

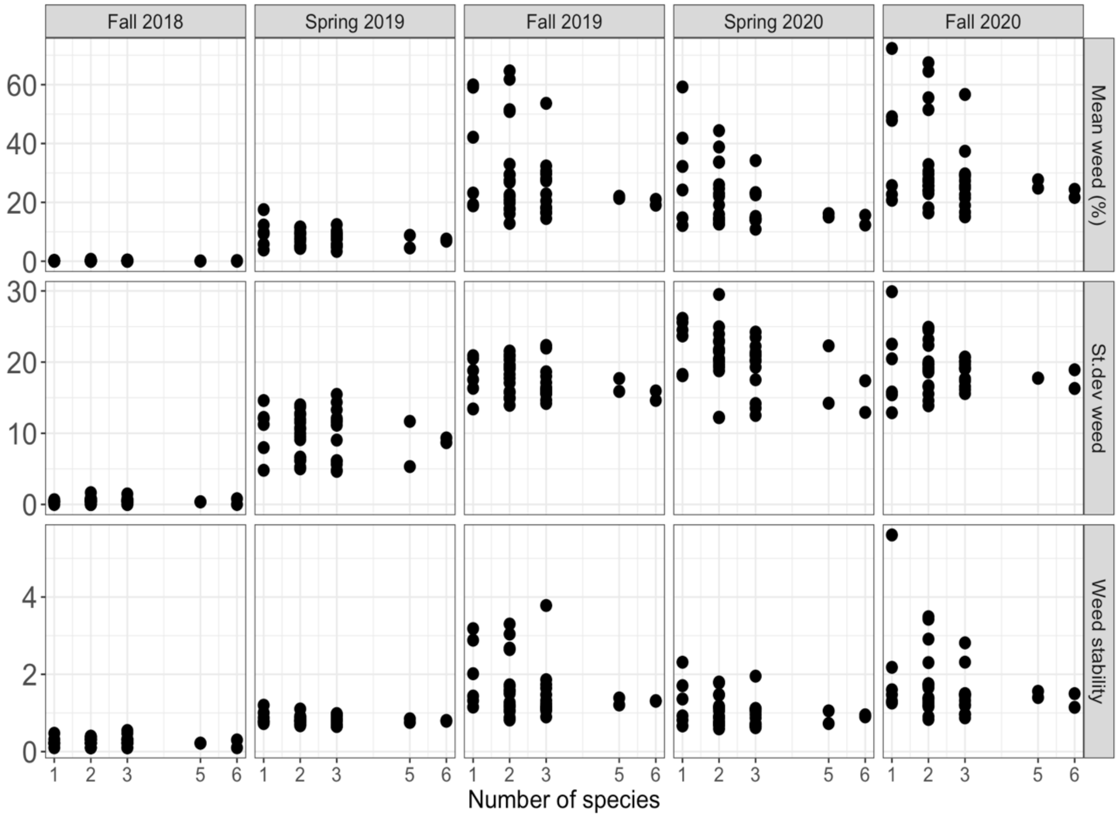

Figure 3.6: Mean, standard deviation, and spatial stability (mean/standard deviation) of weed coverage for each seed treatment for sites seeded in 2019. St. dev = standard deviation. Vegetation sampling season and year are shown facetted in the columns. Undefined stability values changed to 0.1.

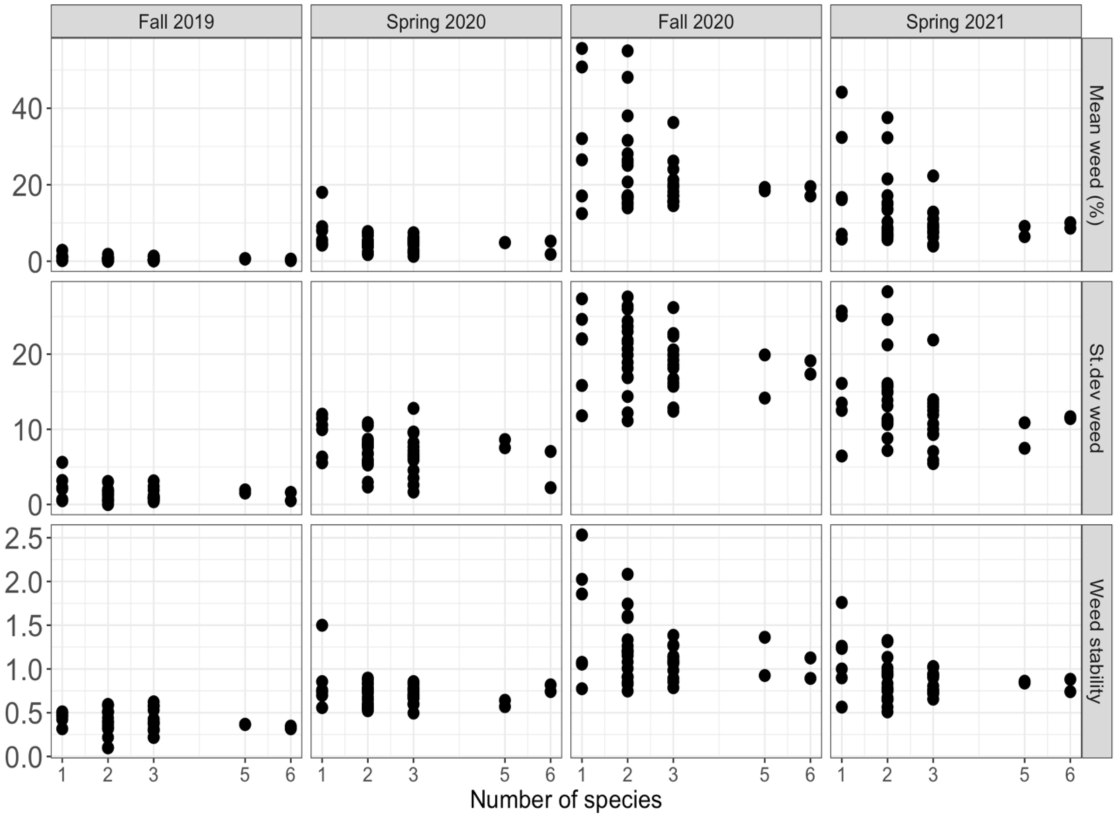

Table 3.9: Linear model with natural log of turfgrass spatial stability. Mean and standard deviation of turfgrass coverage for each seed treatment and time was calculated and then the stability was determined by taking the mean divided by the standard deviation. Coefficient estimates and 95% confidence intervals are back-transformed. There were 360 observations in this analysis.

| | Turfgrass spatial stability | | | |
|---|---|---|---|---|
| | Estimate | Estimate 2.5% | Estimate 97.5% | Pr(>\|t\|) |
| (Intercept) | 1.52521 | 1.33213 | 1.74626 | 2.29E-09 |
| Seeding year 2019 | 1.02453 | 0.94427 | 1.11161 | 0.55950 |
| Season spring | 1.08830 | 1.00359 | 1.18017 | 0.04080 |
| Time | 1.03388 | 1.00229 | 1.06647 | 0.03540 |
| Number of species | 1.08259 | 1.04769 | 1.11866 | 2.80E-06 |

Table 3.10: Linear model with natural log of weed spatial stability plus 0.1. Mean and standard deviation of weed coverage for each seed treatment and time was calculated and then the stability was determined by taking the mean divided by the standard deviation. Coefficient estimates and 95% confidence intervals are back-transformed. There were 360 observations in this analysis.

| | Weed spatial stability | | | |
|---|---|---|---|---|
| | Estimate | Estimate 2.5% | Estimate 97.5% | Pr(>\|t\|) |
| (Intercept) | 0.40924 | 0.34830 | 0.48085 | <2e-16 |
| Seeding year 2019 | 1.01723 | 0.92302 | 1.12105 | 0.72980 |
| Season spring | 0.95088 | 0.86337 | 1.04726 | 0.30560 |
| Time | 1.38544 | 1.33515 | 1.43762 | <2e-16 |
| Number of species | 0.95823 | 0.92154 | 0.99638 | 0.03230 |

# Chapter 4

# Identifying Minnesota Roadside Turfgrass Seeding Clusters based on Different Weather and Soil Factors

## Summary

The Minnesota Department of Transportation (MnDOT) currently recommends two primary statewide turfgrass mixtures for roadsides: one for wetter conditions, and another for drier conditions. The area of Minnesota covers approximately 22,518,133 ha and contains the intersection of three major ecoregions: the boreal forests, deciduous forests, and the prairies. There is substantial variation in climate and soil characteristics across the state, and we hypothesize regional roadside turfgrass mixture recommendations would improve vegetation establishment and persistence. Our objective in this study was to identify if there should be roadside turfgrass seeding clusters, defined by soil and weather variables, to improve turfgrass mixture recommendations. We tested 40 turfgrass seed mixtures that included 6 different species in combinations ranging from monocultures to a 6-way mixture at 14 locations throughout Minnesota. We found differences in soil chemical and physical properties and weather variables among sites. The results of an agglomerative hierarchical cluster analysis identified different clustering solutions. The optimal solution identified two geographical roadside turfgrass seeding clusters (northern, central/southern), and one non-geographical cluster for poor soil quality sites (low organic matter, more sand in soil texture, higher bulk density, and greater saturated paste extract electrical conductivity). Clusters were validated based on the average and standard deviation of turfgrass species coverage at all sites and among clusters. New roadside turfgrass mixture seeding recommendations based on these clusters should result in improved turfgrass establishment and persistence, less erosion, and greater fulfillment of the functions of vegetation along roadsides in Minnesota.

## Introduction

Ideal roadside vegetation and management would reduce the spread of invasive species, reduce dust and glare, prevent erosion, allow for adequate visibility for drivers, and be cost efficient to establish and maintain. Roadside vegetation encompasses an area covering approximately 1% of several states (Forman, 2000), so the type and coverage of roadside vegetation can have a significant impact on the environment and communities.

While MnDOT currently recommends multiple native vegetation mixtures based on region within the state (MacDonagh & Hallyn, 2010), turfgrass seed mixture recommendations are only statewide (MnDOT, 2014) and do not account for the tremendous ecological differences that are found in the state of Minnesota. Some authors have identified the importance of regionally adapted roadside vegetation, but few have tested different mixtures across sites. Tinsley et al. (2006) recommended the need to identify regional adapted native species in Texas. Friell et al. (2015) recommended the examination of different turfgrass mixtures in a wide range of environments and over longer periods of time in Minnesota. Engelhardt (2016) conducted a literature review, and separated four regions in Maryland, and then Engelhardt & Ratliff (2019) went on to test different mixtures within three regions in a field trial, but had difficulty validating regions due to testing at too few research sites. In West Virginia, Hopkinson et al. (2018) assessed if different roadside mixtures are necessary for different elevations and they found that the high elevation site had poorer soils and cooler temperatures, but their proposed high-elevation mixture did not prove to be better than the other mixtures.

Roadside turfgrass experiments have found different species performances in different weather and climate conditions (Brown et al., 2010; Diesburg et al., 1997; Friell et al., 2012; Henslin, 1982; Hottenstein, 1969; Mintenko et al., 2002; White & Smithberg, 1972) and in soil physical and chemical characteristics (Duell & Schmit, 1975; Foote et al., 1978; Henslin, 1982; Hopkinson et al., 2016; Martin & Kaufman, 1970; Watkins et al., 2019). Considering and grouping sites based on both climate and soil characteristics will be referred as clustering, and this form of classification has been widely used in research (Milligan & Cooper, 1987). Clustering could improve roadside turfgrass mixtures recommendations by accounting for some of the most important plant growing factors affecting roadside vegetation.

The state of Minnesota contains variation in temperature, growing degree days, rain and snowfall precipitation, and sunlight. From the latest climate normal (1991-2020), yearly rainfall precipitation can range from 54 to 97 cm. Normal average maximum temperature in July can range from 22.2 to 29.3 ºC, and normal average minimum temperature in January can range from -21.4 to -12.3 ºC. The mean average temperature also ranges from 1.8 to 8.5 ºC (NOAA; MNDNR, State Climatology Office). This variability across the state also has interactions between temperature and climate, since some regions are warmer and dryer, and others cooler and wetter. From these climate differences, regional ecoregions (Omernik, 1987; Omernik & Griffith, 2014), ecosections (MacDonagh & Hallyn, 2010), regional landscape ecosystem zones (Albert, 1994), and provisional seed zones (Bower et al., 2014) have been characterized in Minnesota. These have been based on a variety of soil, climatic, bedrock geology, pre-settlement or potential vegetation, and relative landform, but these may have limited uses for roadside turfgrass adaptability, since a roadside is a highly anthropogenically influenced environment with soils and landforms that are perhaps less likely to be reflective of regional ones. Although, the current recommendation of statewide turfgrass mixtures (MnDOT, 2014) is still likely limiting the long-term persistence of coverage in different areas of the state.

Along with the design of turfgrass mixtures for current climate conditions is the need for a greater appreciation of a mixture withstanding and flourishing in a future climate. Minnesota's latest climate normal (1991-2020) compared to the normal a decade prior (1981-2010) found Minnesota is 6.4 cm wetter in the southern half of the state, and 2.5 cm drier in the northern part of the state. The Minneapolis St. Paul Airport is approximately 0.34 ºC warmer on average. Generally, most of the state is trending warmer and wetter, with only a few areas trending drier (NOAA; MnDNR, State Climatology Office).

A clustering by Ortiz-Valdez (1985) for maize development and Host et al. (1996) for forest resource management are examples of researchers identifying different clusters for regional recommendations and management. Validating clusters can also be challenging, since a few selected sites for a study may not be representative of a whole region (Engelhardt & Ratliff, 2019). Overall, these studies serve as useful templates when considering the clustering of regional roadside turfgrass mixtures in Minnesota. We hypothesize there is enough variation in soils and climate to have different seeding clusters in the state of Minnesota to improve roadside turfgrass recommendations. Therefore, the objective of this research was to identify if there should be different roadside turfgrass seeding clusters incorporating both weather and soil variables in Minnesota, and to validate clustering by assessing the results of an experiment testing turfgrass species and mixtures across the state of Minnesota.

## Materials and methods

### Research sites

Fourteen research sites were selected with an emphasis on locating sites in the 8 MnDOT management regions (Figure 2.1). The result was a uniform spread of sites across Minnesota. Seven were seeded in each of late summer/early fall 2018 and 2019. Site establishment was replicated between years to account for variability in establishment, precipitation, and first-year snowplow and salting management; many of these factors may give rise to short and long-term effects on roadside vegetation. All sites were separated from the road by a standard curb (15.2 cm), except at the Worthington site where the curb was shorter (10.2 cm). Sites were located along a roadway with 2 or 4 total lanes of traffic, and traffic totals were not considered in site selection. Most sites were in full sun, except Edina which contained less morning sunlight due to tree cover, and the first block (repetition) in Chatfield which was partially shaded by an exposed rock face. Prior to site establishment, we noted poor vegetation coverage at both Duluth and Grand Rapids. Public works officials at Duluth noted that several different contractors had attempted to reseed it before with low success (personal communication) while in Grand Rapids, the municipality knew that turfgrass coverage was poor at that site and was therefore given preference for this project (personal communication).

### Additional field methods

The species that were selected, germination testing, treatment and mixture design, soil sampling and testing, supplemental irrigation during establishment, plot maintenance, and data collection can be found in Chapter 3.

### Weather data

An on-site weather station was installed at all sites close to the seeding date and kept at each site until at least the following summer (7 stations in total). Data were recorded by a WatchDog 1400 data logger (Spectrum Technologies, Aurora IL) every thirty minutes. The station recorded soil moisture, temperature, and electrical conductivity using a WaterScout SMEC 300 probe (Spectrum Technologies, Aurora IL) inserted 5 cm into the soil layer. The probe was oriented horizontally at sites seeded in 2018 and vertically at sites seeded in 2019. Average soil moisture was calculated by averaging all available soil moisture data within each location recorded by the SMEC probe. Precipitation was recorded with a tipping bucket and calibrated before installation of sites seeded in 2018. Tipping buckets were subject to clogging from excess debris and values were thus skewed at some sites and not used in further analysis. Labjack Digits-TLH (Sahasra Electronics, India) were also inserted 5 cm into the soil layer in the middle of each block (plot 20, 60, and 100) to monitor soil temperature. Digits recorded soil temperature every 6 hrs. at sites seeded in 2018 and hourly for sites seeded in 2019.

We gathered daily maximum and minimum temperature, rainfall precipitation, snow precipitation, and snow depth from the nearest professionally collected weather station (Table 4.1) (NOAA), since on-site weather stations had inconsistencies in rainfall precipitation and air temperature. A thirty-year climate normal dataset (1991-2020) was calculated for each of these stations to compare observed weather variables during the length of the experiment to climate normals, to show how representative the climate of that research site was to normal conditions. The number of growing degree days was calculated using a base temperature of both 0 and 10 ºC by taking the summation of the sum of the daily maximum and minimum temperature and dividing by two, then subtracting the base temperature.

### Statistics

R software was used for all data processing and analysis (R Core Team, 2021). Average turfgrass coverage, moisture characteristics, and soil physical and chemical characteristics were compared among each research site using a Fisher's least significant difference (LSD) test with the *LSD.test* function in the agricolae package and no p-value correction was applied (de Mendiburu, 2020). To identify distinct roadside seeding clusters, we gathered all weather and soil variables that are thought to have a significant effect on turfgrass coverage on roadsides over time. Weather variables collected from the on-site station included minimum winter soil temperature, number of days of soil temperature below -5 ºC, and number of days of soil temperature above 35 ºC. Weather variables collected from the nearest professionally collected weather station were the cumulative sum of the total number of growing degree days at base 0 ºC and 10 ºC, average maximum temperature (TMAX), average minimum temperature (TMIN), sum of rainfall precipitation (PRCP), sum of snowfall precipitation (SNOW), and average snow depth (SNWD) all from weather observed in 2020. Latitude (LAT) was included as a proxy for potential sunlight quantity. The soil variables, which were averaged for each site, included the average moisture from the on-site station (Table 4.2); potential plant available water (Table 4.2); sand, silt, clay, and organic matter (OM); saturated paste extract electrical conductivity (SEC); total and fine bulk density (Table 4.3); and porosity. Elevation was not included because Minnesota is a relatively flat landscape. In total, 21 weather and soil variables were used as input criteria to distinguish turfgrass seeding clusters in the state of Minnesota.

Scatterplots of all weather and soil variables were examined and a correlation matrix was tested with all pairwise comparisons. If a correlation was greater than 0.95, then the variable considered secondary was removed; for instance, temperature and growing degree days were highly correlated, but growing degree days is calculated from temperature, so growing degree days was removed from the analysis. Some correlations between 0.9-0.95 were kept and others were discarded. We did not include soil heavy metal information to avoid less relevant variables, which could lead to false site differentiation (Milligan & Cooper, 1987). We attempted to use soil temperature variables in the cluster analysis, but winter snow removal at some sites and inconsistent logging intervals did not allow for uniform comparison. The final 12 variables that remained to be tested in the cluster analysis were TMAX, TMIN, PRCP, SNOW, SNWD, and LAT for weather variables, and sand, clay, OM, SEC, and total and fine bulk density for soil variables. After all variables were collected, they were scaled and centered using the *scale* function in R for cluster analysis.

A principal components analysis was used to plot weather and soil variables at different research sites using the *rda* function in the vegan package with scaling set to true (Oksanen et al., 2020). An agglomerative hierarchical cluster analysis (Milligan & Cooper, 1987) was applied to identify significant seeding clusters using the *hclust* function with the ward.D2 clustering method (Ward, 1963). This was applied on the results of the distance matrix using the *dist* function in R with the Euclidean method. Both *hclust* and *dist* are in the stats package incorporated into base R (R Core Team, 2021). An additional cluster analysis was tested using only the weather variables to identify how that clustering scenario would contrast with both weather and soil variables. The *NbClust* function in the NbClust package (Charrad et al., 2014) was used to identify the optimal number of clusters with the Hubert (Hubert & Arabie, 1985) and Dindex (Lebart et al., 1995) graphical indices. The optimal number of clusters were validated by examining and comparing the coverage and standard deviation of species monocultures between clusters and research sites.

## Results

### Soil physical and chemical characteristics

Research sites differed in soil physical and chemical properties (Table 4.3). Most sites were classified as a sandy clay loam. Grand Rapids contained an average organic matter content of 1.8% and was similar to Bemidji at 2.1%, Duluth at 2.6%, and Chatfield and St. Cloud both at 2.7%. International Falls and Edina contained the most organic matter at 6.6% and 5.8%, respectively. Duluth contained the lowest available K (56 mg kg$^{-1}$) similar to Grand Rapids, Edina, Roseville, Brainerd, and Bemidji. The saturated paste extract electrical conductivity was the highest for Duluth (1.63 mmhos cm$^{-1}$), Willmar (1.53 mmhos cm$^{-1}$), Edina (1.43 mmhos cm$^{-1}$), and St. Cloud (1.4 mmhos cm$^{-1}$). Content of Zn (43.3 mg kg$^{-1}$) and Cu (14.4 mg kg$^{-1}$) were both greater at the Brainerd site than all other locations, likely due to its proximity to a nearby metal recycling plant: truck traffic from the recycling plant was known to deposit dust along the road, which subsequently accumulated onto the roadside. Grand Rapids contained the highest fine bulk density (1.58 g cm$^{-3}$) and both Grand Rapids and Duluth contained the two highest total bulk densities at 1.71 g cm$^{-3}$ and 1.61 g cm$^{-3}$, respectively (Table 4.3).

### Weather and climate differences

In general, research sites in the southern part of the state experienced warmer maximum and minimum temperatures (Table 4.4), and generally more growing degree days (Table 4.5). Sites located in southern Minnesota also experienced the potential for more intense sunlight based on the latitude. Rainfall precipitation in 2020 was the highest for Chatfield at 794 mm and lowest for East Grand Forks with 464 mm. The snowfall precipitation in 2020 was highest at Duluth (2127 mm), due to lacustrine effects, and lowest for Fergus Falls (726 mm) (Table 4.4).

The deviation in observed weather for the duration of the experiment and the climate normal (1991-2020) by month for all research sites are shown in Figures 4.1-4. For all sites, one contained a cooler temperature maximum than normal in 2020, and 4 contained a cooler temperature minimum than normal (Table 4.6). In 2020, the greatest deviations in average maximum temperature were found in a warmer Fergus Falls (1.1 ºC) and a cooler Roseville (-0.5ºC) (Table 4.6). Deviation in observed precipitation from climate normals found most sites were drier than normal (Table 4.6). Duluth received less rain than expected in the month of June (-94 mm), and Grand Rapids observed more rain than normal in August (+138 mm) (Fig. 4.3).

Soil temperature using the SMEC probe was highly variable across the research sites (Fig. 4.5). Duluth peaked at a higher temperature than at all other sites with 15 days of maximum soil temperature greater than 40 ºC (data not shown). Duluth had even greater soil temperature extremes than Worthington, despite Worthington containing a warmer yearly average air temperature (Table 4.4), but Worthington had 53 days of average soil temperature greater than 30 ºC, whereas Duluth had only 38 days above that threshold. The soil temperature at Duluth also exhibited high thermal conductivity with a range of 55.4 ºC in 2020 using the SMEC probe, and in the months of June, July, and August 2020 the average daily soil temperature range fluctuated 15.6 ºC (Fig. 4.5).

## Cluster solutions

Correlations between final variables that were used for the cluster analysis are shown in Table 4.7. The principal components analysis plot shows the ordination distribution of the 14 research locations from the 12 weather and soil variables (Fig. 4.6). The dendrogram plot shows the results of the hierarchical cluster analysis beginning with 14 distinct clusters (for n number of research sites) to 1. Research sites closer to one another are more similar within the same branch (Fig. 4.7). A two-to-six seeding cluster solution from the dendrogram plot is shown in Table 4.8.

A two seeding cluster solution would distinguish Bemidji, Brainerd, Duluth, and Grand Rapids from the others. Based on the principal components plot, those four research sites are in a similar ordination space with higher sand content, greater bulk density, higher saturated paste extract electrical conductivity, and less organic matter, along with more snowfall and greater snow depth. If three seeding clusters are distinguished, then East Grand Forks and International Falls become the next cluster; these are both located in northern Minnesota and the sites contain more organic matter and clay and experience lower temperatures. Four seeding clusters would another group consisting of Marshall and Worthington, both located in southwest Minnesota and each site had similar organic matter content, less sand, and warmer temperatures. At four seeding clusters, the remaining sites still clustered together are located from southeast to central Minnesota. A fifth seeding cluster separates Duluth from Bemidji, Brainerd, and Grand Rapids. A sixth seeding cluster would separate the central Minnesota locations (Fergus Falls, Saint Cloud, and Willmar) from the more southeast locations (Edina, Chatfield, and Roseville). The optimum number of clusters for the dendrogram based on the Hubert and Dindex indices was three (Fig. 4.8). If only the weather variables were used to cluster the sites, then East Grand Forks and International Falls were found to cluster together as the most similar sites within a branch (data not shown), similar initially to the clustering solution shown in Figure 4.7, but then the next similar site was Duluth in this scenario. Based on the observed species composition at Duluth (Fig. 4.14-16), differences exist in individual species and total turfgrass coverage between itself and East Grand Forks and International Falls.

### Cluster validation

The mean and standard deviation of species monoculture coverage within a cluster approximately one year after seeding compared to all research sites is shown in Table 4.9. Research sites that were classified as poor soil quality sites contained the lowest average cluster coverage one year after seeding (30%). Total average observed turfgrass coverage at Brainerd, Duluth, Bemidji, and Grand Rapids was 18.5%, 25.4%, 38.8%, and 45.6%, respectively. Brainerd and Duluth additionally had the poorest average turfgrass coverage before the first winter, which was only 7.9% and 23.5%, respectively. The northern and central/southern cluster contained similar total average turfgrass coverage but have differences in the type of coverage (Table 4.9). The northern cluster compared to the central/southern cluster had more alkaligrass, less buffalograss, less tall fescue, and relatively similar amounts of hard fescue, Kentucky bluegrass, and slender creeping red fescue (Table 4.9).

Both hard fescue and slender creeping red fescue, the two fine fescue species, were the top performing monoculture species for all clusters. One difference between slender creeping red fescue and hard fescue is that slender had a higher standard deviation for the central/southern cluster compared to hard fescue. Buffalograss coverage was the most abundant at poor soil quality sites (41%) and within that cluster it was only greater than Kentucky bluegrass and tall fescue (Table 4.9). Tall fescue contained the most coverage at research sites classified in the central/southern cluster (74%) and the lowest coverage at the poor soil quality sites (17%), and within the poor soil quality cluster, tall fescue was one of the poorest performers compared to the other monoculture treatments. Tall fescue also had the highest standard deviation compared to the other monoculture species across all research sites (Table 4.9). Alkaligrass had the highest average coverage at poor soil quality sites (27%) and at that cluster was the only monoculture species comparable all others. Alkaligrass was also similar to buffalograss as the poorest performing monoculture species (Table 4.9). Kentucky bluegrass had better coverage at the northern and central/southern cluster than the poor soil quality cluster (Table 4.9).

The standard deviation of an individual monoculture was less for all clusters compared to all research sites except for one instance; in that instance, the standard deviation of alkaligrass was higher at poor soil quality sites (Table 4.9). Based on the reduction in standard deviation for 17 of 18 monoculture species in the three-cluster scenario, our statistical approach has distinguished the unique strengths and weaknesses of different species based on soil and weather differences and shows to be a valid method of clustering. Therefore, we validated that the optimum solution was a three-cluster scenario, with clusters for (1) northern Minnesota, (2) central/southern Minnesota, and (3) sites throughout the state with poor soil characteristics.

## Discussion

There are currently only statewide recommended seed mixtures for roadsides in Minnesota; differences in seed mixture components are largely based on roadside aesthetics rather than geographic or edaphic factors (MnDOT, 2014). We gathered 12 variables and clustered sites based on those differences and validated the clustering results by assessing our turfgrass coverage and deviation (Fig. 4.9-26; Table 4.9). Based on our cluster analysis results, we recommend three seeding clusters for Minnesota (Fig. 4.7-8; Table 4.8): two geographic clusters (northern and central/southern), and one cluster based on poor soil characteristics of a site (Fig. 4.15-16; 4.24-26; Table 4.9).

### Soil characteristics and remediation

Soil characteristics of sites classified within the poor soil quality cluster include high sand and low clay contents, low organic matter, high bulk density, and high saturated paste extract electrical conductivity relative to sites contained within the geographical clusters (Fig. 4.6; Table 4.3). A previous roadside research experiment covering multiple states found that an urban site in New Jersey with the poorest coverage contained the highest saturated paste extract electrical conductivity (8.7 mmhos $cm^{-1}$) (Watkins et al., 2019), and only weeping and seaside alkaligrass [*Puccinellia maritima* (Huds.) Pari.] contained statistically more than 0% coverage at that site two years after seeding. This shows the importance of using specific species for sites with high saturated paste extract electrical conductivity. Hopkinson et al. (2016) evaluated vegetation cover at 29 roadsides and medians in West Virginia and found sites containing less than 50% vegetation cover were associated with poor soil properties. Poor soil properties were defined as containing high saturated paste extract electrical conductivity ranging from 0.36-1.54 mmhos $cm^{-1}$, or low organic matter content of 1.7% or less. Additionally, Hopkinson et al. (2016) found that soil texture was not a significant factor, and an organic matter content of 2.2% was recommended as sufficient for roadsides. With this organic matter criterion, Grand Rapids and Bemidji, in our study, would have limited vegetation cover potential, assuming a species that was seeded could not tolerate a lower threshold. Vegetation coverage is also more limited when bulk density is greater than 1.7 g $cm^{-3}$ on coarser soils (Daddow, 1983) and this could have limited coverage at Grand Rapids and portions of Duluth. In West Virginia, Hopkinson et al. (2018) tested different roadside mixtures at different elevations and found less total coverage at the high-elevation site, and those findings were attributed to poor soil conditions.

The soil texture can also have a significant impact on coverage. Greater sand content in soils has been found to allow for greater hydraulic conductivity when the soil is frozen (Stoeckeler & Weitzman, 1960). In Minnesota, roadsides are salted in the winter and since roadside soils are usually sandier, then the combination could allow more salt damage. One experiment conducted by Haan et al. (2012) found more sand in the soil resulted in improved overwintering of forbs, but those findings may not be applicable to turfgrass. Baadshaug (1973) found that a sandy loam soil texture was colder by 0.5-1 ºC in the winter than a clay texture. Baadshaug (1973) also found a sandy loam was the second worst texture for spring grass coverage on plots that contained an ice sheet treatment and those that were snow-free. Clay soils resulted in the greatest injury on snow free plots, but the least amount on ice-covered plots, therefore it is important to recognize that winter effects and soil physical factors are complex (Baadshaug, 1973). Recommending a turfgrass seed mixture for specific soil characteristics would not be unusual then with the limitations that are common among roadside soils.

We found lower average coverage for the poor soil quality cluster (Table 4.9) suggesting that there is opportunity for improved species and cultivar recommendations and/or soil remediation. A cost-tradeoff would be helpful to identify if and how much topsoil or compost should be incorporated into a site depending on the current soil conditions. Mixtures could be evaluated in soil containing different levels of remediation, thereby allowing for a non-dichotomous decision. Duell & Schmit (1975) found that amending a roadside site containing 96% sand with 5 cm of silty clay topsoil could improve establishment. In a similar manner, Dunifon et al. (2011) found good turfgrass establishment for one year when applying compost; however, coverage began to decline 1-2 years after seeding, and those findings were attributed to poor subsurface soil conditions, in addition to high compaction. Watkins & Trappe (2017) found no benefits of applying several different amendments along roadsides, but those results could have been limited by the tested sites containing sufficient soil chemical and physical characteristics.

### Species and cluster coverage differences

There was evidence of slender creeping red fescue with a larger standard deviation in the central/southern cluster compared to the northern cluster (Table 4.9). We observed ‘SeaMist’ slender creeping red fescue was less adapted to the warmer regions than ‘Gladiator’ hard fescue, especially in the middle of the summer and if it was dry, potentially explaining that larger variation. Tall fescue was found to contain the largest standard deviation of all monoculture species showing that in some instances in can be a large benefit along roadsides and in others, such as in the poor soil quality cluster, in can be of little benefit. Tall fescue is known to be susceptible to winter injury in Minnesota along roadsides (Friell et al., 2015) explaining why it may have performed the best in the warmer areas of the state (Table 4.4; Table 4.9) and ones with warmer winter soil temperature (Fig. 4.5). Duluth was likely heavily affected by soil properties compared to weather variables and illustrates the importance of clustering by both soil and weather variables, since with weather variables alone, the clustering of Duluth with East Grand Forks and International Falls would not be appropriate, since species and total coverage was much different (Fig. 4.14-16).

### Research site management

Total amount and type of coverage can also be impacted by the management of research sites. We observed cooler winter minimum soil temperature at sites that had aboveground snowfall cleared in the winter months (Chatfield, Duluth, Bemidji, Brainerd, Roseville, and possibly more) (Fig. 4.5). Sites with natural snow cover tended to have more turfgrass coverage the following spring, and this could be due to soil temperatures hovering around 0 ºC, especially at Marshall and Edina (Fig. 4.5). This indicates the need to, when possible, maintain some snow cover on recently seeded roadside vegetation.

This reflects similar findings in a grass survival experiment conducted by Baadshaug (1973). Bemidji also experienced lower coverage due to direct winter snowplow damage in addition to the indirect winter effects from less snow cover. Other factors such as the mowing height and frequency could also alter soil temperature, which could influence the amount of light that reaches the surface. Collectively, this shows that soil temperature is a dynamic variable not only influenced by the climate and edaphic factors of the area, but also by management factors dictating its peaks and variation in a single day to an entire season.

### Limitations

Based on our research site selection, we may have oversampled stretches of roadsides that are difficult to maintain vegetation. This was the case based on a few cities giving preference to these areas. Site selection shows some limitations based on the results of our principal components analysis plot (Fig. 4.6). We are lacking sites containing both high clay content and high bulk density, since root limitations are significant at 1.4 g $cm^{-3}$ or greater for clay soils (Daddow, 1983). We are also lacking a site located in southwest Minnesota (Fig. 2.1) containing soil properties similar to sites classified within the poor soil quality cluster. Maintaining similar mowing within a couple sites and across all sites was also difficult and this could have affected species performance disproportionately. Data collection only occurred in the fall and in the spring each year and differences were observed in species coverage especially in the middle of the growing season at some sites.

## Conclusion

Based on data collected from 14 research sites, we have identified three different turfgrass seeding clusters for Minnesota. One cluster was designated for poor soil quality sites and two are geographical clusters. Future research should focus on defining a poor soil quality site, and the regional clusters could be expanded, refined, or modified. Future turfgrass mixtures testing in Minnesota will need to consider the trend of climate changes in a region. Species and mixtures should be recommended that are tolerant of current and future conditions. Continued consideration should be given to identify species and cultivars that are adapted to roadsides and the cluster of interest. Germplasm could be collected along roadsides and tested in the respective cluster for adaptability. Together, this will continue to create headway to improve the establishment and persistence of turfgrasses over time along Minnesota roadways. This will reduce erosion and visibility impairments and maintain a safer roadway.

Table 4.1: The nearest weather station to each roadside research site. Data collected from each station began when it was seeded and contained the temperature maximum, minimum, rainfall and snowfall precipitation, and snow depth. The station chosen was the nearest one with the least missing data.

| Research site | Station name | Station ID | City | State[a] | Latitude | Longitude |
|---|---|---|---|---|---|---|
| Grand Rapids | Pokegama, MN US | USC00216612 | Grand Rapids | MN | 47.2508 | -93.5861 |
| Grand Rapids[5] | Grand Rapids Frs Lab, MN US | USC00213303 | Grand Rapids | MN | 47.2436 | -93.4975 |
| Brainerd | Brainerd, MN US | USC00210939 | Brainerd | MN | 46.3433 | -94.2086 |
| Brainerd[6] | Brainerd Crow Wing Co Airport, MN US | USW00094938 | Brainerd | MN | 46.40472 | -94.13083 |
| E. G. Forks | Grand Forks University NWS, ND, US | USC00323621 | Grand Forks | ND | 47.92172 | -97.0975 |
| Fergus Falls[1] | Orwell Dam, MN US | USC00216228 | Fergus Falls | MN | 46.2154 | -96.178 |
| Fergus Falls[2] | Breckenridge 3 E, MN US | USC00210974 | Breckenridge | MN | 46.3047 | -92.5216 |
| Roseville | University of MN St. Paul, MN US | USC00218450 | St. Paul | MN | 44.9902 | -93.17995 |
| Marshall | Marshall, MN US | USC00215204 | Marshall | MN | 44.4716 | -95.79019 |
| Chatfield | Rochester International Airport, MN US | USW00014925 | Rochester | MN | 43.9041 | -92.4916 |
| Bemidji[3] | Bemidji, MN US | USR0000MBEM | Bemidji | MN | 47.5032 | -94.9281 |
| Bemidji[4] | Bemidji, MN US | USC00210643 | Bemidji | MN | 47.5353 | -94.8268 |
| Int. Falls | Int. Falls International Airport, MN US | USW00014918 | International Falls | MN | 48.5614 | -93.3981 |
| Duluth | Duluth International Airport, MN US | USW00014913 | Duluth | MN | 46.8369 | -92.1833 |
| Saint Cloud | St. Cloud Regional Airport, MN US | USW00014926 | Saint Cloud | MN | 45.5433 | -94.0513 |
| Willmar | Willmar 5 N, MN US | USC00219001 | Willmar | MN | 45.1901 | -95.0586 |
| Edina[7] | Minneapolis St. Paul International Airport, MN US | USW00014922 | Minneapolis | MN | 44.8831 | -93.2289 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Worthington | Worthington 2 NNE, MN US | USC00219170 | Worthington | MN | 43.6449 | -95.5802 |

[1,2]Orwell is much closer but was missing data from Jan. 8-31, 2019, therefore Breckenridge weather data was used to fill that gap. [3]Missing precipitation data. [4]Missing temperature data. [5]Used only to fill in Aug. 2019 and Sep. 2020 missing weather data. [6]Used to fill in Jan. 2021 missing temperature data. Brainerd still missing January snowfall and depth data. [7]Edina weather data appears more skewed from urban heat island effect than other sites.

[a] MN = Minnesota, ND = North Dakota.

Table 4.2: Average soil moisture and other moisture characteristics based on or affected by the soil properties for each site (Saxton et al., 1986).

| Research site | Average moisture (%)[ab] | Field capacity (%)[b] | Wilting point (%)[b] | Potential plant available water (%)[b] |
|---|---|---|---|---|
| Bemidji | 6.98 k | 22.7 i | 13.7 i | 8.99 hi |
| Brainerd | 4.31 l | 23 i | 14.5 ghi | 8.58 i |
| Chatfield | 13 g | 28.6 ef | 18.3 e | 10.4 efg |
| Duluth | 7.27 j | 25.1 gh | 14.1 hi | 11 de |
| E. G. Forks | 18.4 e | 44 a | 27.6 a | 16.4 a |
| Edina | 20.8 c | 26.9 fg | 15.4 fgh | 11.6 cd |
| Fergus Falls | 10.2 h | 28.4 ef | 17.5 e | 10.9 def |
| G. Rapids | 9.65 i | 24.3 hi | 14.1 hi | 10.2 efg |
| Int. Falls | 15.4 f | 32.6 d | 20.7 d | 11.9 bc |
| Marshall | 21.3 b | 36.1 c | 23.5 c | 12.7 b |
| Roseville | 20 d | 25.2 gh | 15.6 fg | 9.6 gh |
| Saint Cloud | 13.1 g | 26 gh | 16 f | 9.99 fg |
| Willmar | 21.3 b | 30.1 e | 17.9 e | 12.2 bc |
| Worthington | 24.9 a | 40.7 b | 25.1 b | 15.6 a |

[a] Average moisture calculated from all available moisture data points available for each site. This results in a different number of sampling data points per site.

[b] Significant differences are based on Fisher's LSD with no p-value correction applied.

Table 4.3: Average soil chemical and physical characteristics at each research site. Bulk density values are averaged from samples taken at three different distances from the curb within each of the three blocks. Significant differences are based on Fisher's LSD with no p-value correction applied.

| Research site | K[c] | OM[b] | Sat. elect. conductivity (mmhos $\text{cm}^{-1}$) | Sand (%) | Clay (%) | Soil textural class[a] | Zn[c] | Cu[c] | Fine bulk density (g $\text{cm}^{-3}$) | Coarse bulk density (g $\text{cm}^{-3}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Bemidji | 82 e | 2.07 fg | 1.07 bc | 69.6 a | 20 f | SCL | 2.84 f | 0.344 d | 1.38 b | 1.43 b |
| Brainerd | 78.3 e | 3.1 def | 0.733 cde | 71.2 a | 21.7 f | SCL | 43.3 a | 14.4 a | 1.34 b | 1.4 b |
| Chatfield | 133 d | 2.7 efg | 0.767 cde | 52.5 cd | 31.7 d | SCL | 1.43 f | 0.379 d | 1.22 cde | 1.28 cd |
| Duluth | 56 e | 2.6 efg | 1.63 a | 55 bcd | 22.1 f | SCL | 3.74 def | 4.7 b | 1.31 bc | 1.61 a |
| E. G. Forks | 240 a | 4.97 bc | 0.633 de | 3.93 i | 47.5 a | Silty clay | 1.71 f | 1.4 cd | 1.11 efg | 1.14 ef |
| Edina | 69.7 e | 5.8 ab | 1.43 ab | 49.2 de | 25.4 e | SCL | 6.74 c | 1.11 cd | 1.28 bcd | 1.35 bc |
| Fergus Falls | 231 a | 5.1 bc | 0.967 cd | 50.4 d | 30 d | SCL | 6.17 cd | 1.15 cd | 1.19 def | 1.28 cd |
| Grand Rapids | 59.3 e | 1.83 g | 0.8 cde | 60 b | 21.7 f | SCL | 2.95 f | 1.03 cd | 1.58 a | 1.71 a |
| Int. Falls | 129 d | 6.63 a | 1.1 bc | 41.2 f | 36.7 c | CL | 3.49 ef | 1.19 cd | 0.95 h | 1.05 f |
| Marshall | 203 ab | 4 cd | 0.467 e | 34.4 g | 42.1 b | Clay | 3.41 ef | 1.19 cd | 1.1 fg | 1.18 de |
| Roseville | 74.3 e | 5.13 b | 0.633 de | 61.7 b | 25 e | SCL | 12.8 b | 2.59 c | 1.23 cd | 1.27 cd |
| Saint Cloud | 142 cd | 2.7 efg | 1.4 ab | 58.3 bc | 26.3 e | SCL | 2.42 f | 0.532 d | 1.36 b | 1.43 b |
| Willmar | 183 bc | 3.43 de | 1.53 a | 42.5 ef | 31.3 d | CL | 5.97 cde | 1.02 cd | 1.29 bcd | 1.36 bc |
| Worthington | 170 bcd | 5.03 bc | 0.833 cde | 16.3 h | 44.2 b | Clay | 2.88 f | 1.01 cd | 1.05 gh | 1.11 ef |

[a] SCL = sandy clay loam, CL = clay loam.

[b] OM = organic matter content.

[c] Units of mg $\text{kg}^{-1}$.

Table 4.4: Observed yearly weather summary totals and means from weather stations. Not showing snow depth metrics.

| | 2018[c] | | | | 2019[c] | | | | 2020 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Research site | TMAX[a] | TMIN[a] | PRCP[b] | SNOW[b] | TMAX | TMIN | PRCP | SNOW | TMAX | TMIN | PRCP | SNOW |
| Bemidji | | | | | 6.3 | -2.6 | 382 | 1141 | 10.8 | -1.0 | 723 | 1287 |
| Brainerd | 4.8 | -4.2 | 214 | 558 | 9.5 | -2.1 | 991 | 2067 | 11.0 | -1.2 | 780 | 1316 |
| Chatfield | 5.6 | -2.8 | 267 | 390 | 10.9 | 1.2 | 1403 | 2342 | 12.7 | 2.4 | 794 | 1275 |
| Duluth | | | | | 6.1 | -1.6 | 363 | 1430 | 10.3 | 0.1 | 541 | 2127 |
| E. G. Forks | 3.0 | -5.4 | 151 | 599 | 8.6 | -1.4 | 862 | 2509 | 11.2 | -0.3 | 464 | 1006 |
| Edina | | | | | 9.4 | 1.7 | 307 | 653 | 13.6 | 4.1 | 759 | 1432 |
| F. Falls | 4.6 | -5.7 | 153 | 625 | 9.4 | -1.5 | 759 | 1457 | 11.6 | -0.5 | 599 | 726 |
| G. Rapids | 5.2 | -3.1 | 245 | 204 | 9.0 | -2.4 | 781 | 1638 | 10.6 | -1.6 | 653 | 979 |
| Int. Falls | | | | | 5.7 | -3.1 | 356 | 768 | 10.3 | -2.8 | 546 | 1553 |
| Marshall | 8.0 | -3.8 | 309 | 513 | 11.8 | 0.1 | 1164 | 2197 | 14.1 | 1.3 | 587 | 1183 |
| Roseville | 5.9 | -1.7 | 286 | 293 | 10.7 | 1.4 | 1091 | 2212 | 12.4 | 2.6 | 656 | 1247 |
| St. Cloud | | | | | 5.3 | -3.6 | 229 | 592 | 12.5 | 1.1 | 680 | 1142 |
| Willmar | | | | | 6.0 | -2.8 | 251 | 566 | 12.1 | 1.0 | 559 | 895 |
| Worthin. | | | | | 8.4 | -1.2 | 349 | 436 | 13.1 | 1.7 | 578 | 1255 |

[a] Average for each site within each year is in units of (°C). TMAX = maximum temperature, TMIN = minimum temperature.

[b] Sum for each site within each year in units of (mm). PRCP = rainfall precipitation, SNOW = snowfall precipitation.

[c] Research sites seeded in 2018 and 2019 showcase the means and sums of what each site received, so not necessarily a full year of weather data, but beginning when the site was seeded.

Table 4.5: Total number of yearly observed cumulative growing degree days at base 0 °C and 10 °C for each site beginning by seeding date from the nearest weather location.

| | 2018 | | 2019 | | 2020[a] | | 2021[b] | |
|---|---|---|---|---|---|---|---|---|
| Research site | 0 °C | 10 °C | 0 °C | 10 °C | 0 °C | 10 °C | 0 °C | 10 °C |
| Bemidji | 0 | 0 | 696 | 177 | 2902 | 1120 | 550 | 115 |
| Brainerd | 411 | 89 | 2878 | 1081 | 2765 | 1054 | 601 | 127 |
| Chatfield | 421 | 65 | 3294 | 1337 | 3461 | 1435 | 783 | 180 |
| Duluth | 0 | 0 | 617 | 156 | 2871 | 1084 | 541 | 100 |
| E. G. Forks | 326 | 43 | 2999 | 1198 | 3164 | 1365 | 553 | 97 |
| Edina | 0 | 0 | 908 | 330 | 3796 | 1699 | 831 | 205 |
| Fergus Falls | 348 | 63 | 2969 | 1151 | 3143 | 1313 | 83 | 0 |
| G. Rapids | 576 | 155 | 2773 | 993 | 2875 | 1125 | 528 | 111 |
| Int. Falls | 0 | 0 | 638 | 136 | 2650 | 944 | 507 | 102 |
| Marshall | 483 | 79 | 3434 | 1488 | 3651 | 1654 | 746 | 181 |
| Roseville | 511 | 123 | 3334 | 1364 | 3481 | 1497 | 324 | 79 |
| Saint Cloud | 0 | 0 | 432 | 101 | 3315 | 1371 | 653 | 139 |
| Willmar | 0 | 0 | 555 | 164 | 3337 | 1412 | 650 | 153 |
| Worthington | 0 | 0 | 733 | 241 | 3504 | 1492 | 202 | 22 |

[a] First year to compare total growing degree days between all 14 sites.

[b] Not a complete year of experienced weather data so this column is missing values disproportionately by site.

Figure 4.1: Monthly climate normal (1991-2020) temperature maximum data subtracted by observed monthly average. Redder indicates a location was warmer than normal and bluer indicates it was cooler than normal. TMAX = maximum temperature.

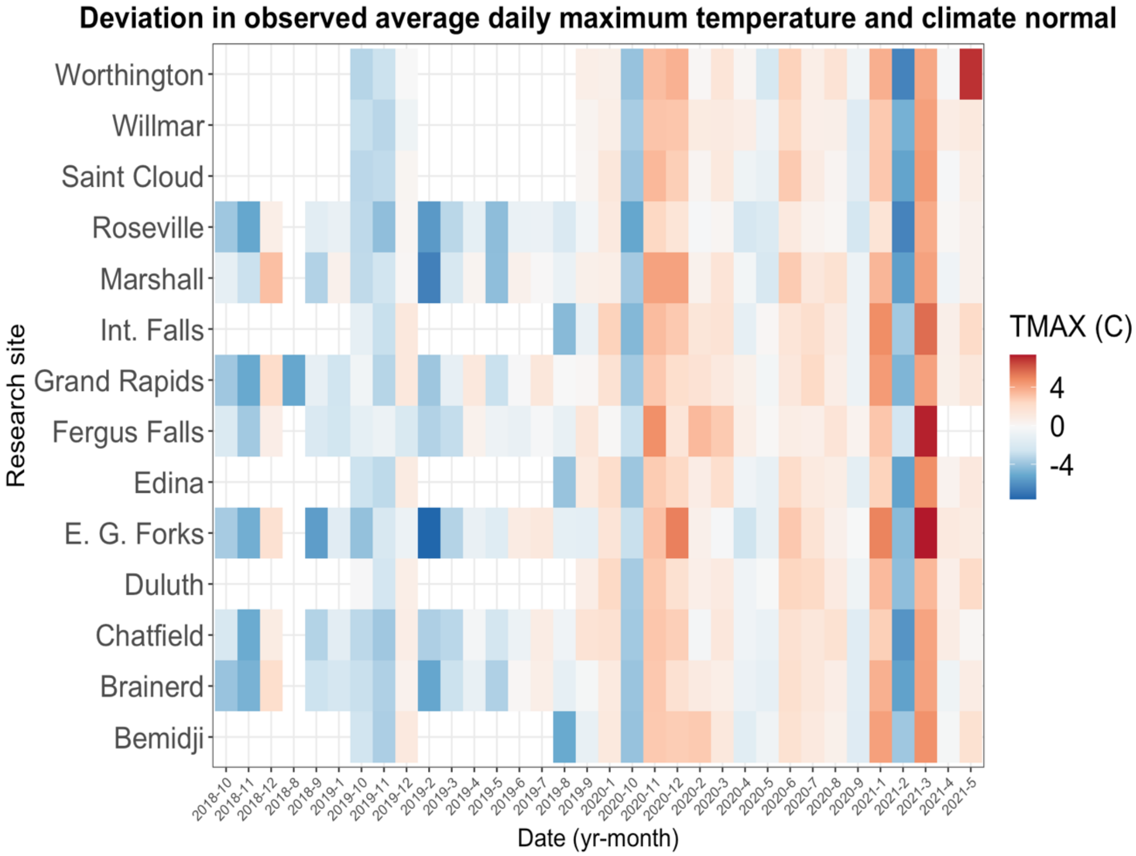

Figure 4.2: Monthly climate normal (1991-2020) temperature minimum data subtracted by observed monthly average. Redder indicates a location was warmer than normal and bluer indicates it was cooler than normal. TMIN = minimum temperature.

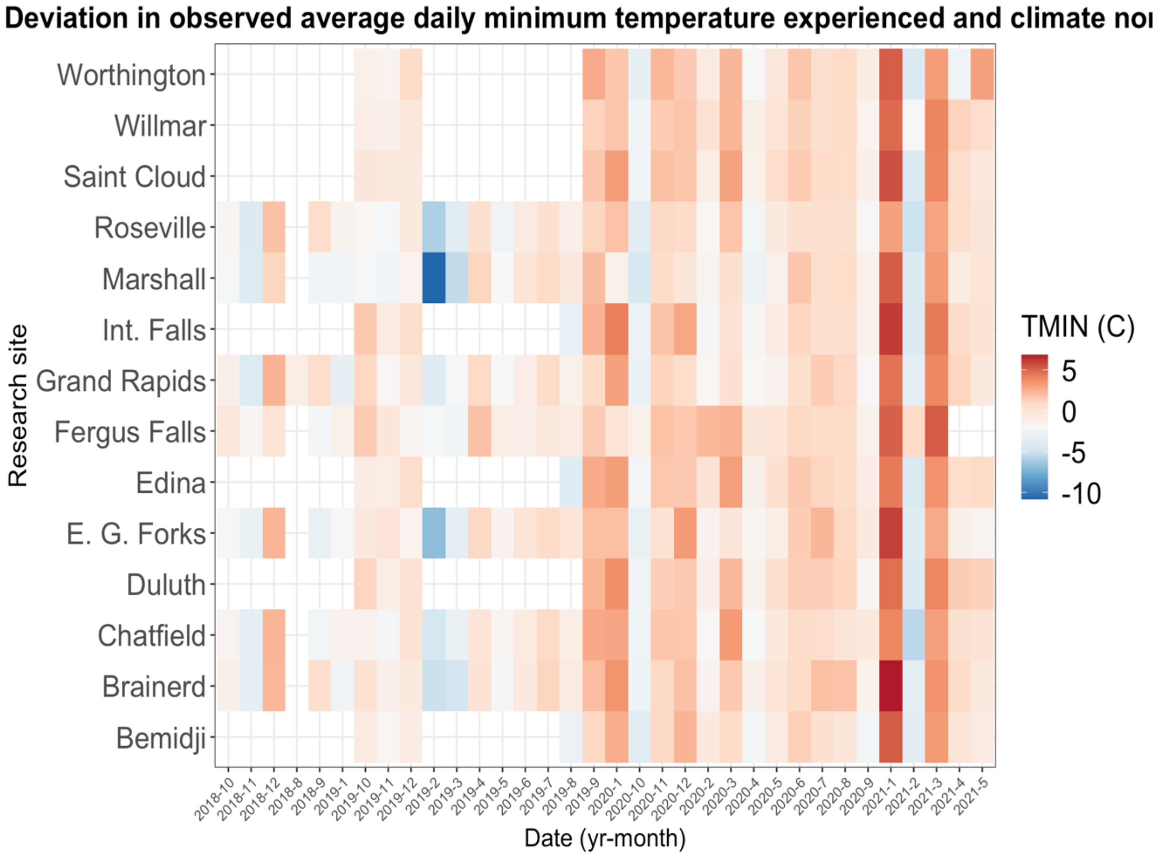

Figure 4.3: Monthly climate normal (1991-2020) rainfall precipitation data subtracted by experienced monthly average. Redder indicates a location was drier than normal and green indicates it was wetter than normal. PRCP = rainfall precipitation.

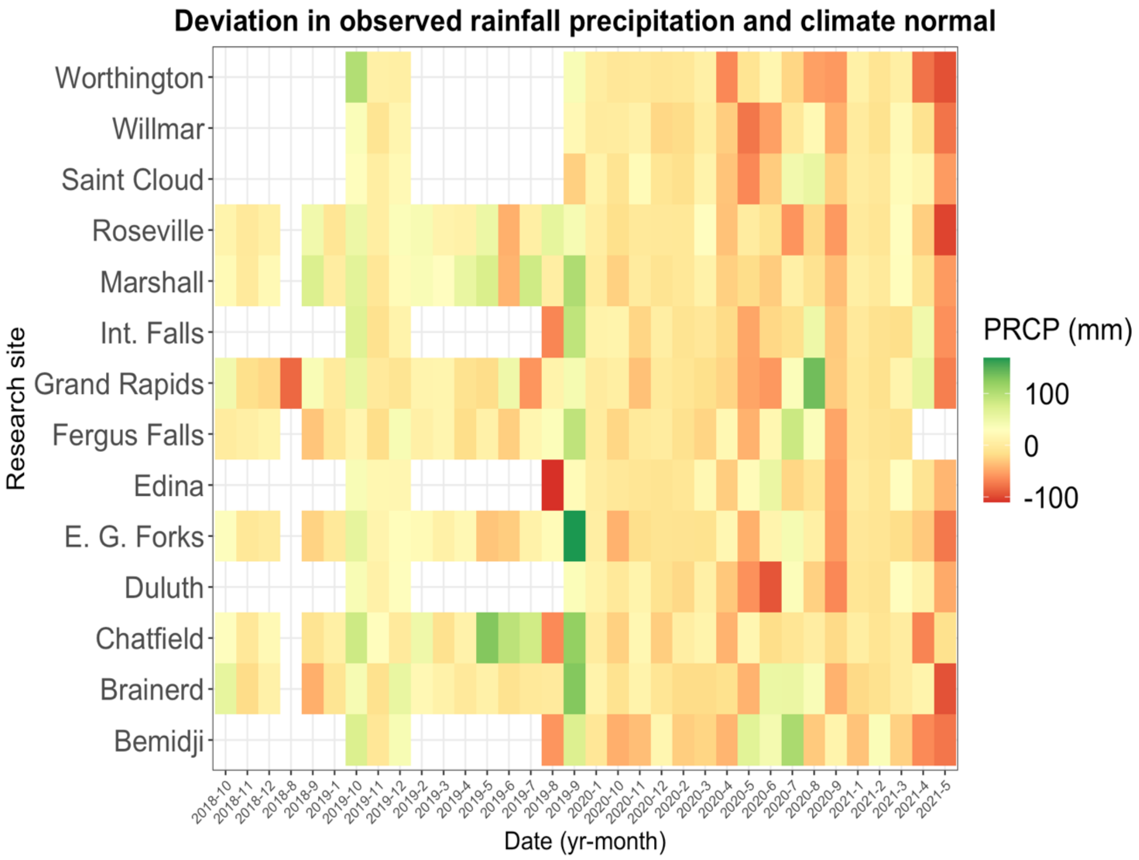

Figure 4.4: Monthly climate normal (1991-2020) snowfall precipitation data subtracted by experienced monthly average. Browner indicates a location had less snowfall than normal and greener indicates a location had more snow than normal in that month. SNOW = snowfall precipitation.

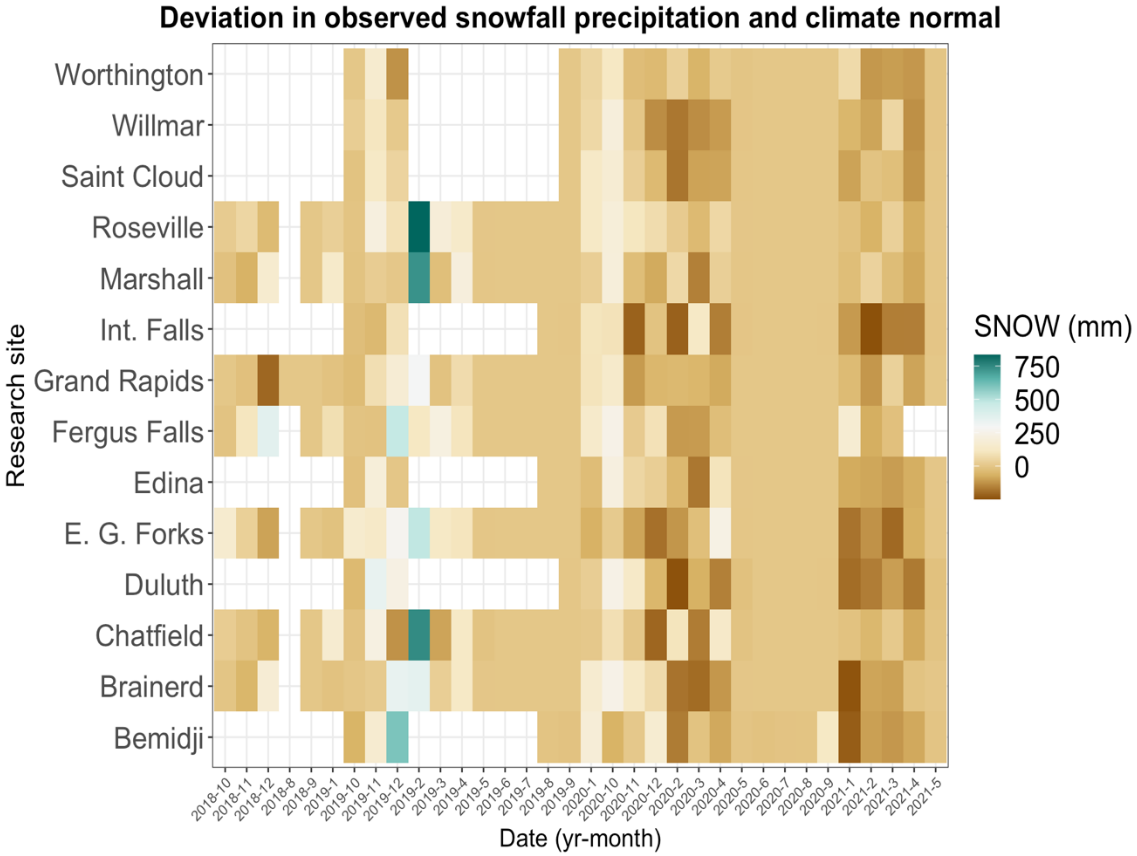

Table 4.6: Deviation in observed weather data (in 2020) and climate normals (1991-2020) for each research site. The source of the weather data is from the nearest station. More negative values represent cooler temperatures, less rainfall, or less snowfall precipitation than normal (1991-2020). TMAX = maximum temperature, TMIN = minimum temperature, PRCP = rainfall precipitation, SNOW = snowfall precipitation.

| | Deviation between observed and expected climate normal | | | |
|---|---|---|---|---|
| Research site | TMAX (°C)[a] | TMIN (°C)[a] | PRCP (mm)[b] | SNOW (mm)[b] |
| Bemidji | 0.52 | -0.11 | 33.4 | 92.9 |
| Brainerd | 0.30 | 0.50 | 8.0 | 92.4 |
| Chatfield | 0.50 | 0.28 | -86.8 | -66.9 |
| Duluth | 0.78 | 0.53 | -251.0 | -165.0 |
| E. G. Forks | 0.70 | 0.20 | -112.0 | -259.0 |
| Edina | 0.58 | 0.77 | -43.7 | 129.0 |
| Fergus Falls | 1.12 | 0.68 | 0.04 | 233.0 |
| G. Rapids | 0.77 | -0.12 | -46.4 | -108.0 |
| Int. Falls | 0.81 | 0.15 | -98.4 | -303.0 |
| Marshall | 0.79 | -0.73 | -148.0 | 33.5 |
| Roseville | -0.46 | -0.27 | -179.0 | 515.0 |
| Saint Cloud | 0.45 | 0.63 | -43.5 | -89.5 |
| Willmar | 0.58 | 0.42 | -216.0 | -309.0 |
| Worthington | 0.59 | 0.31 | -205.0 | 64.4 |

Figure 4.5: Average monthly soil temperature using the SMEC probe. All data was recorded every thirty-minutes. International Falls is missing data from Oct. 18, 2019 to Feb. 12, 2020 and Sep. 14, 2020 to Feb. 15, 2021. Temperatures of close to -40 ℃ caused loggers to malfunction. Duluth is missing data from Mar. 6-May 21, 2019. Edina SMEC probe failed to record data beginning on Oct. 13th, 2020.

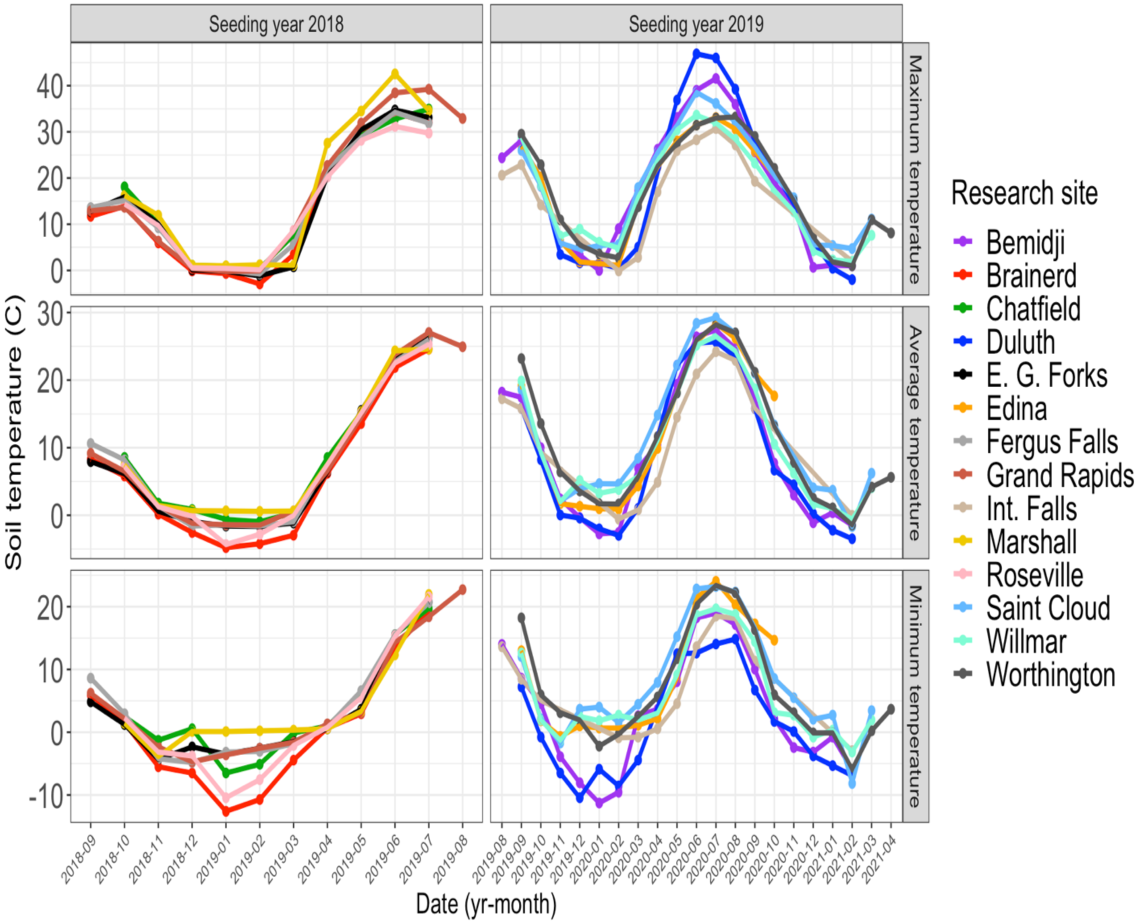

Table 4.7: Correlation matrix of remaining climate and soil variables used in the cluster analysis and ordination plotting. LAT = latitude, SNWD = snow depth, SNOW = snowfall precipitation, SEC = saturated paste extract electrical conductivity, OM = organic matter content, BD = bulk density, PRCP = rainfall precipitation, TMAX = temperature maximum, TMIN = temperature minimum.

| | TMAX | TMIN | PRCP | SNOW | SNWD | SAND | CLAY | OM | SEC | Total BD | Fine BD | LAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TMAX | 1.000 | | | | | | | | | | | |
| TMIN | 0.833 | 1.000 | | | | | | | | | | |
| PRCP | 0.228 | 0.305 | 1.000 | | | | | | | | | |
| SNOW | -0.230 | 0.022 | 0.026 | 1.000 | | | | | | | | |
| SNWD | -0.774 | -0.636 | -0.024 | 0.473 | 1.000 | | | | | | | |
| SAND | -0.234 | -0.080 | 0.710 | 0.158 | 0.317 | 1.000 | | | | | | |
| CLAY | 0.311 | 0.030 | -0.617 | -0.214 | -0.390 | -0.939 | 1.000 | | | | | |
| OM | 0.332 | 0.280 | -0.321 | -0.084 | -0.483 | -0.547 | 0.552 | 1.000 | | | | |
| SEC | -0.168 | 0.122 | -0.021 | 0.359 | 0.191 | 0.232 | -0.414 | -0.162 | 1.000 | | | |
| Total BD | -0.356 | -0.115 | 0.308 | 0.156 | 0.398 | 0.663 | -0.818 | -0.751 | 0.417 | 1.000 | | |
| Fine BD | -0.245 | -0.047 | 0.467 | -0.085 | 0.309 | 0.688 | -0.817 | -0.738 | 0.281 | 0.940 | 1.000 | |
| LAT | -0.866 | -0.820 | -0.340 | 0.123 | 0.724 | 0.052 | -0.118 | -0.100 | 0.074 | 0.166 | 0.097 | 1.000 |

Figure 4.6: Results of the principal components plot. A three cluster dendrogram overlay connects selected sites. LAT = latitude, SNWD = snow depth, SNOW = snowfall precipitation, SEC = saturated paste extract electrical conductivity, OM = organic matter content, BD = bulk density, PRCP = rainfall precipitation, TMAX = temperature maximum, TMIN = temperature minimum. Principal components plot was plotted with scaling set to true.

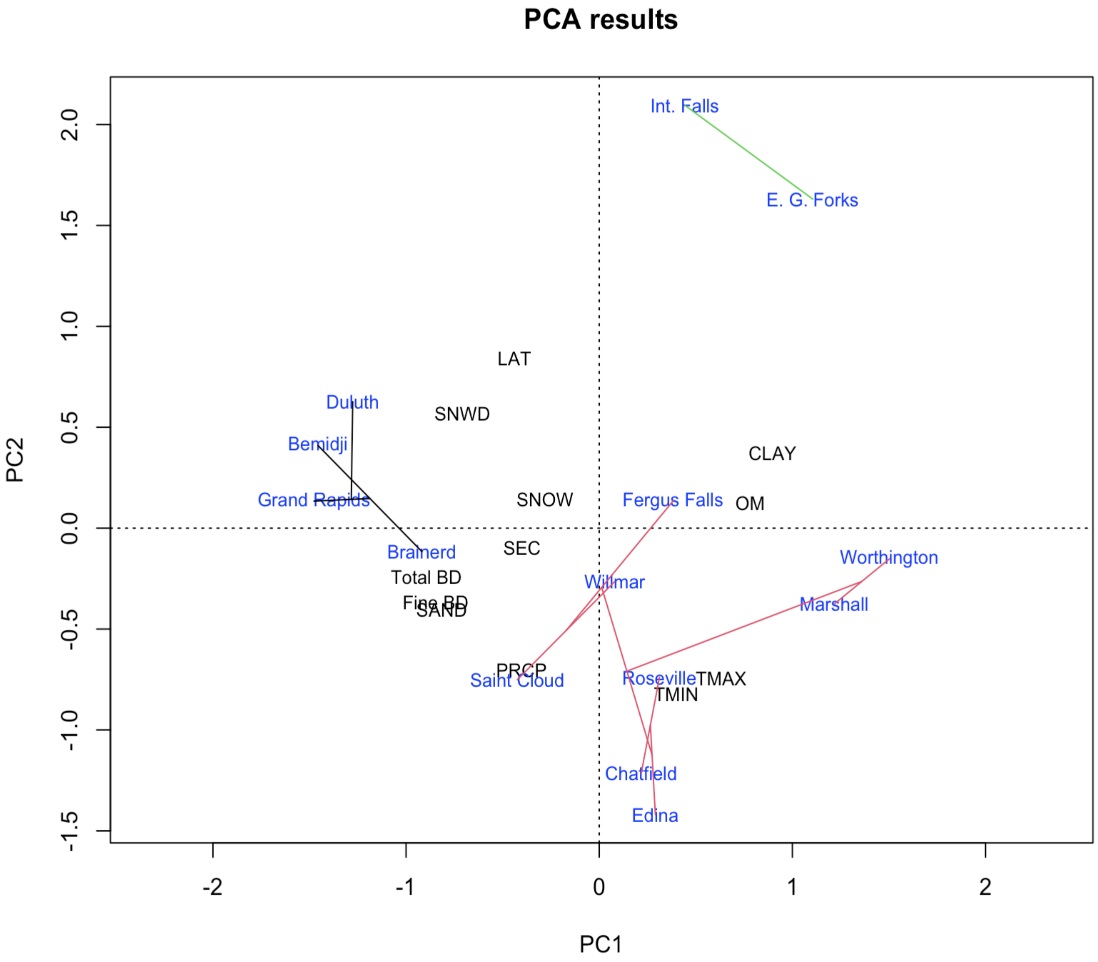

Figure 4.7: Hierarchical agglomerative cluster dendrogram plot. Clustering is based on 12 weather and soil variables. Beginning with 14 research sites (at the bottom), the sites most similar group together, and additional branches within a branch are most similar. The number of significant seeding clusters depends on what height the tree is pruned at.

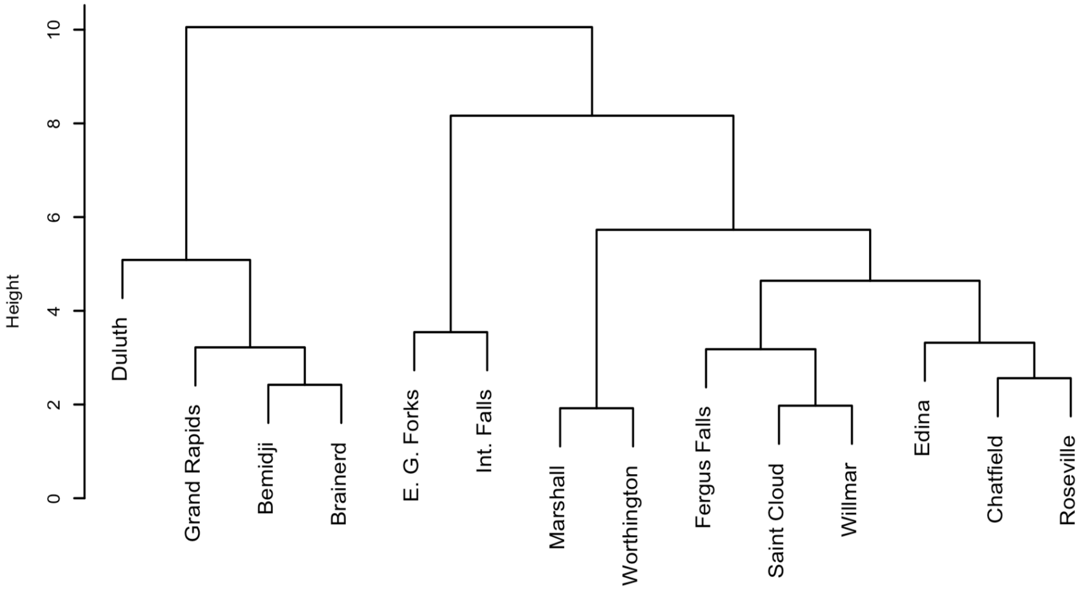

Table 4.8: Depicting how different sites are clustering. This table shows the same information as in the dendrogram plot, except that only 6 seeding clusters are shown instead of 14.

| Number of seeding clusters | | | | |
|---|---|---|---|---|
| 2 | 3 | 4 | 5 | 6 |
| Bemidji | Bemidji | Bemidji | Duluth | Duluth |
| Brainerd | Brainerd | Brainerd | Bemidji | Bemidji |
| Duluth | Duluth | Duluth | Brainerd | Brainerd |
| Grand Rapids | Grand Rapids | Grand Rapids | Grand Rapids | Grand Rapids |
| Chatfield | E. G. Forks | E. G. Forks | E. G. Forks | E. G. Forks |
| E. G. Forks | Int. Falls | Int. Falls | Int. Falls | Int. Falls |
| Edina | Chatfield | Marshall | Marshall | Marshall |
| Fergus Falls | Edina | Worthington | Worthington | Worthington |
| Int. Falls | Fergus Falls | Chatfield | Chatfield | Fergus Falls |
| Marshall | Marshall | Edina | Edina | Saint Cloud |
| Roseville | Roseville | Fergus Falls | Fergus Falls | Willmar |
| Saint Cloud | Saint Cloud | Roseville | Roseville | Chatfield |
| Willmar | Willmar | Saint Cloud | Saint Cloud | Edina |
| Worthington | Worthington | Willmar | Willmar | Roseville |

Figure 4.8: Graphical plots of Dindex and Hubert's optimum clustering selection. The peak in the second difference plot shows the optimal number of seeding clusters. Both statistical approaches identify three clusters.

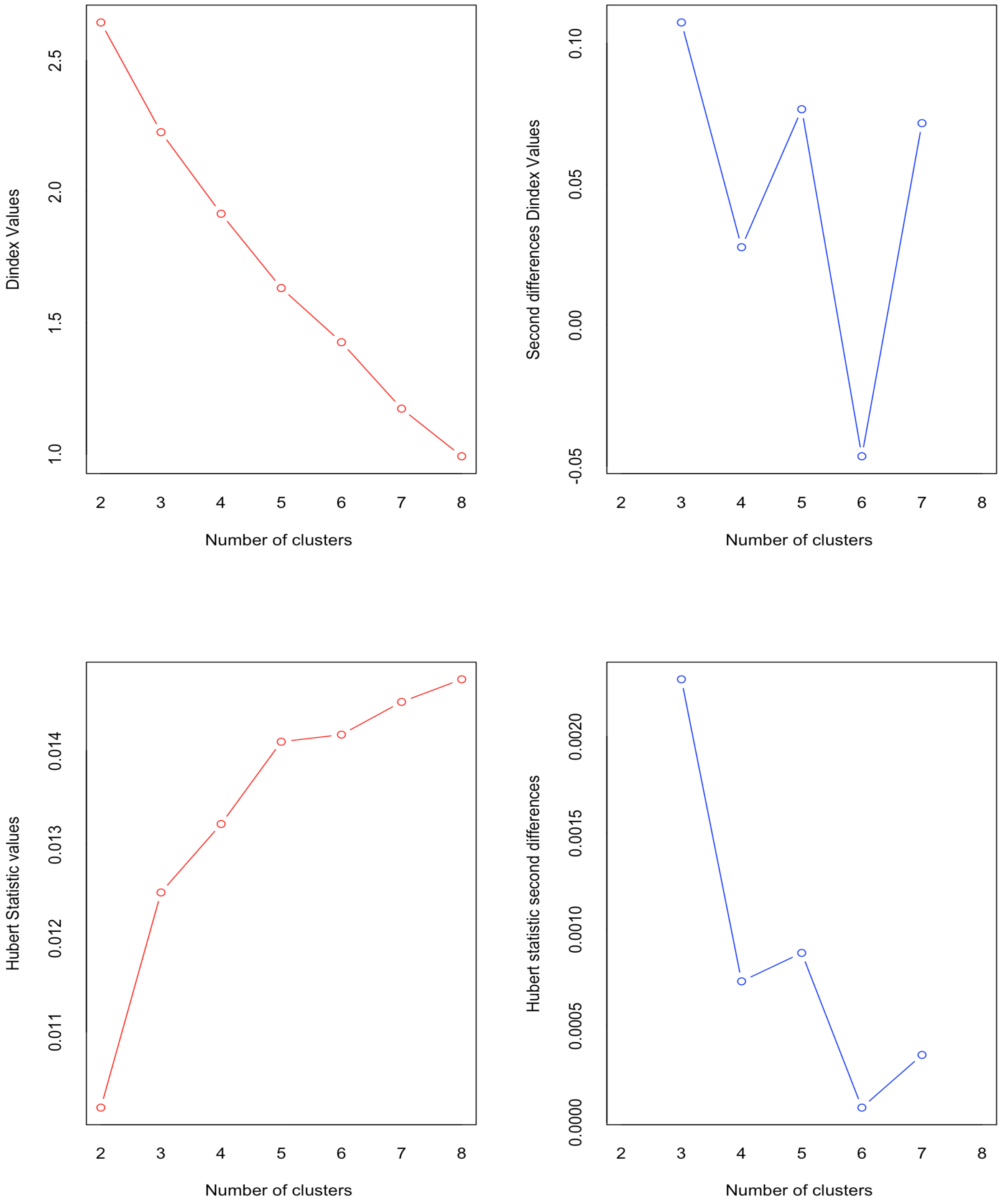

Figure 4.9: Turfgrass coverage sampling for poor soil quality cluster sampled the first fall. Grand Rapids and Brainerd sampled in fall 2018, and Bemidji and Duluth sampled in fall 2019. Brainerd had poor establishment likely due to the site being the only one irrigated by a water truck, instead of the modular drip irrigation system and it contains the highest sand content at 71% (Table 4.3). The brown dashed line is at 70% coverage and allows the viewer to quickly compare treatments between sites. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

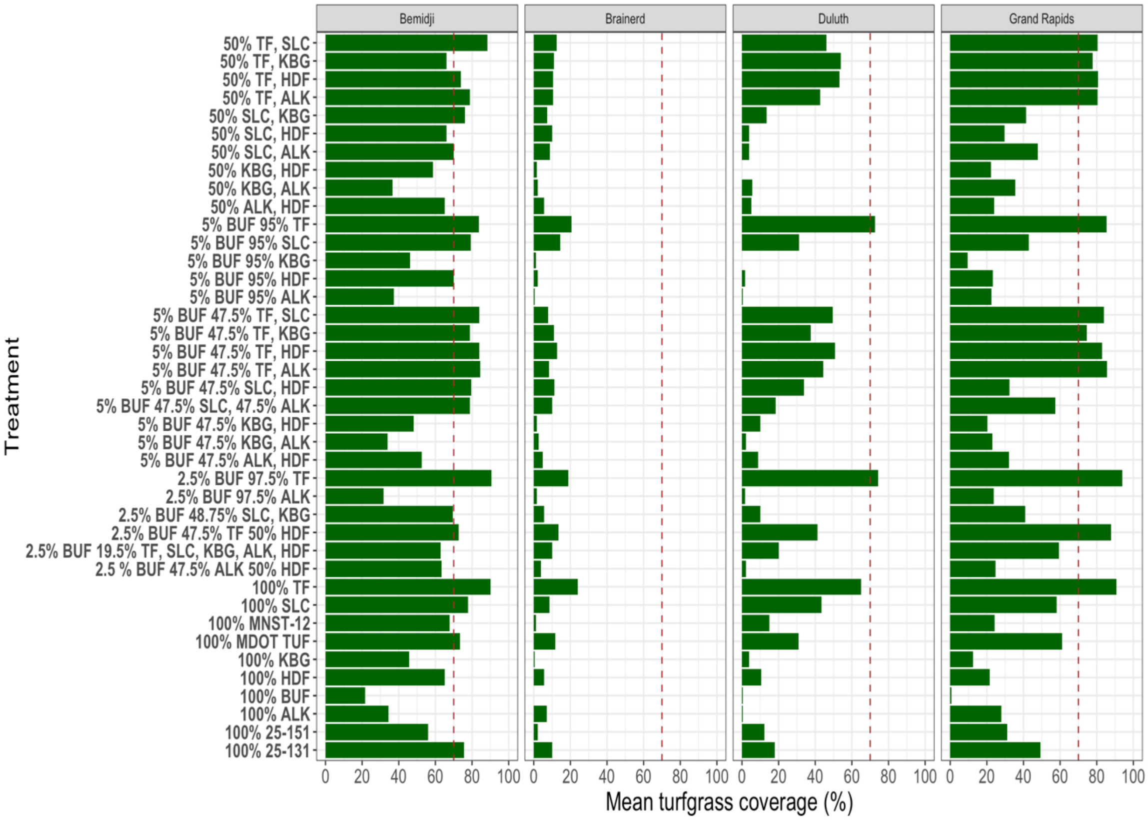

Figure 4.10: Turfgrass coverage sampling for poor soil quality cluster sampled in the spring after the first fall. Grand Rapids and Brainerd sampled in spring 2019, and Bemidji and Duluth sampled in spring 2020. Some plots at Bemidji, especially within blocks one and two were significantly impacted by direct plow damage in plots reducing coverage by approximately 5-20% and indirect effects from extremely cold temperatures (Fig. 4.5), so greater standard deviation can be expected in treatment coverage at that location.TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

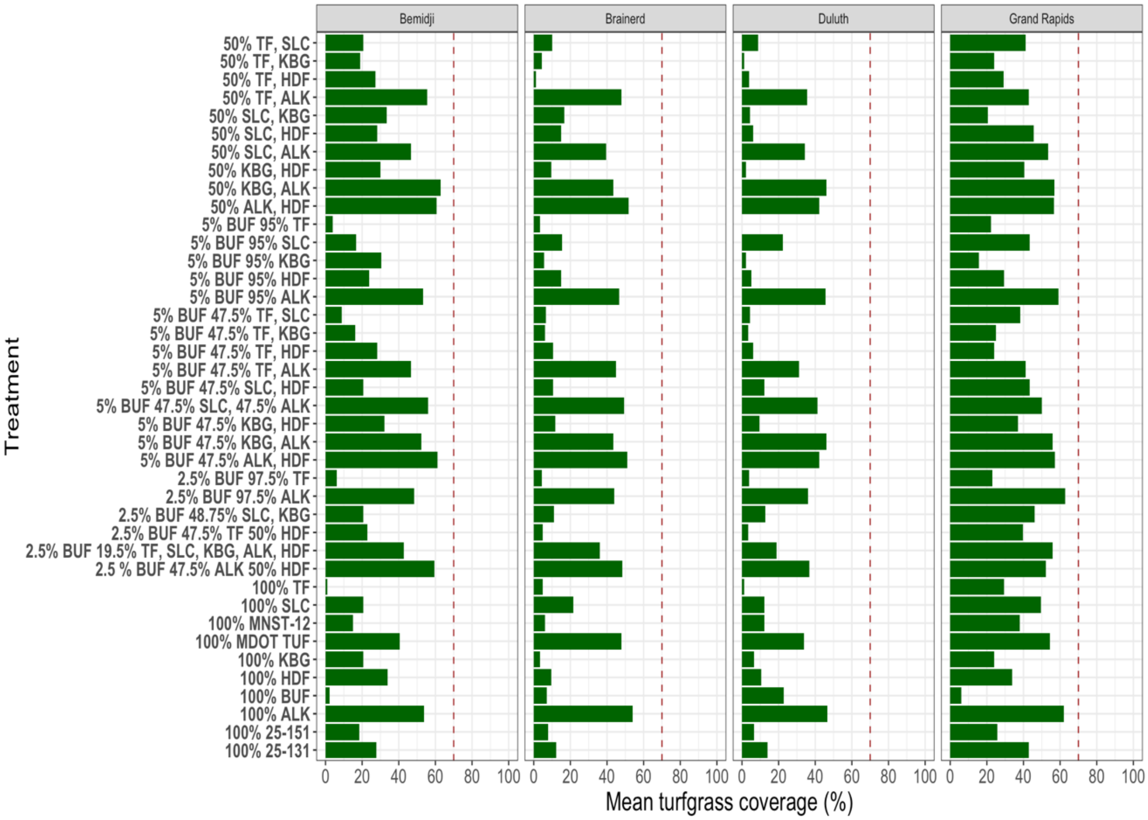

Figure 4.11: Turfgrass coverage sampling for first fall (1) and spring (2) with the sites clustered in the north. East Grand Forks was sampled in fall 2018 (1) and spring 2019 (2). International Falls was sampled in fall 2019 (1) and then spring 2020 (2). East Grand Forks had the lowest growing degree days prior to the first freeze after seeding in the fall of 2018 (233 and 41, base 0ºC and 10ºC, respectively (data not showing)); this may explain its low coverage going into the first winter. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

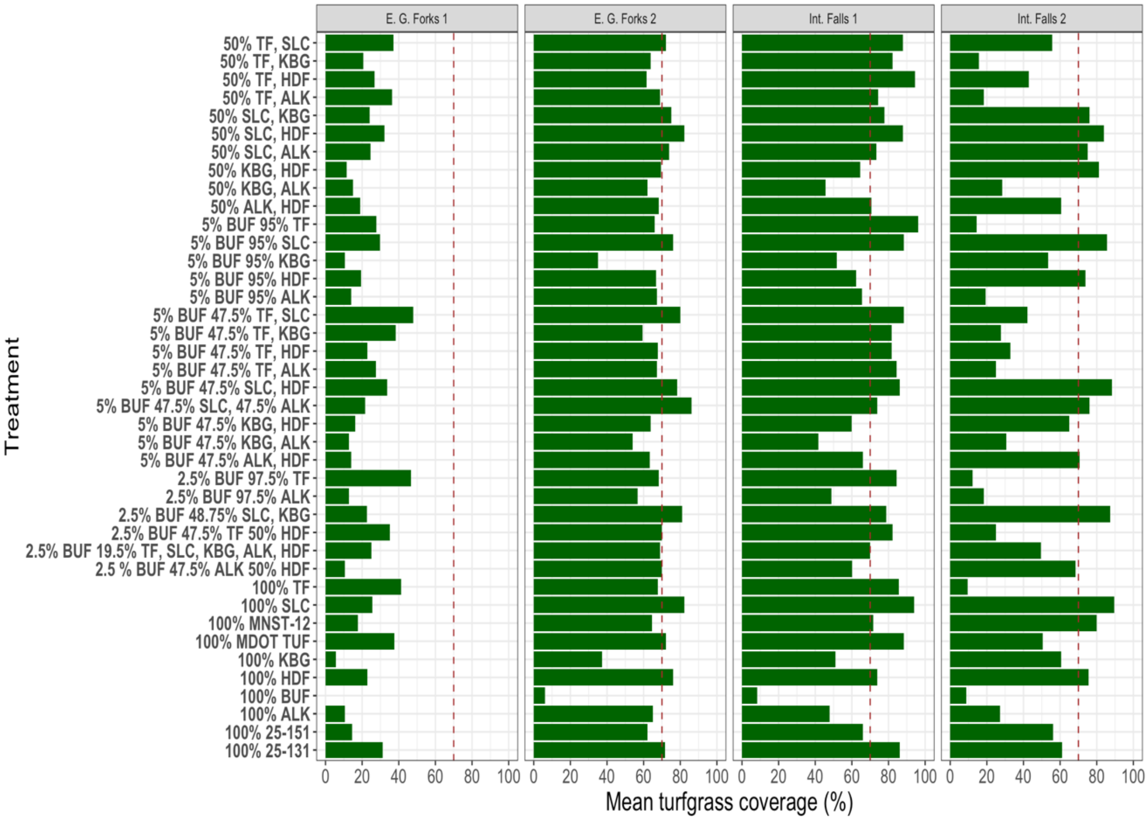

Figure 4.12: Turfgrass coverage sampling in the first fall for the seeding cluster located in central to southeast Minnesota. Fergus Falls had the second fewest total growing degree days in fall 2018 before winter with 348 and 63 base 0 ºC and 10 ºC, respectively, and was sampled that fall with even lower growing degree days of 273 and 63, base 0 ºC and 10 ºC, respectively (data not shown). TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

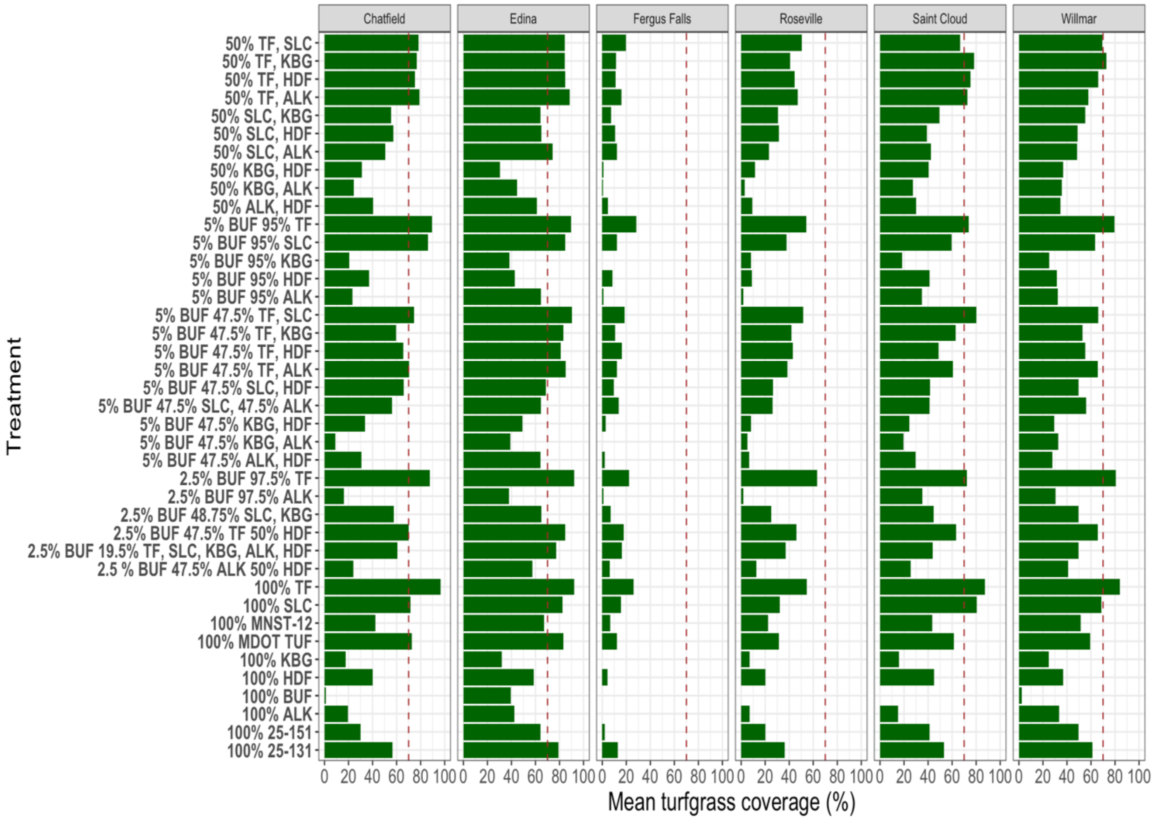

Figure 4.13: Turfgrass coverage sampling in the first spring after fall for the seeding cluster located in central to southeast Minnesota. Snow was known to be cleared from Chatfield and Roseville in the first winter, and this indirect affect was observed to cause significant damage to tall fescue coverage at Roseville. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

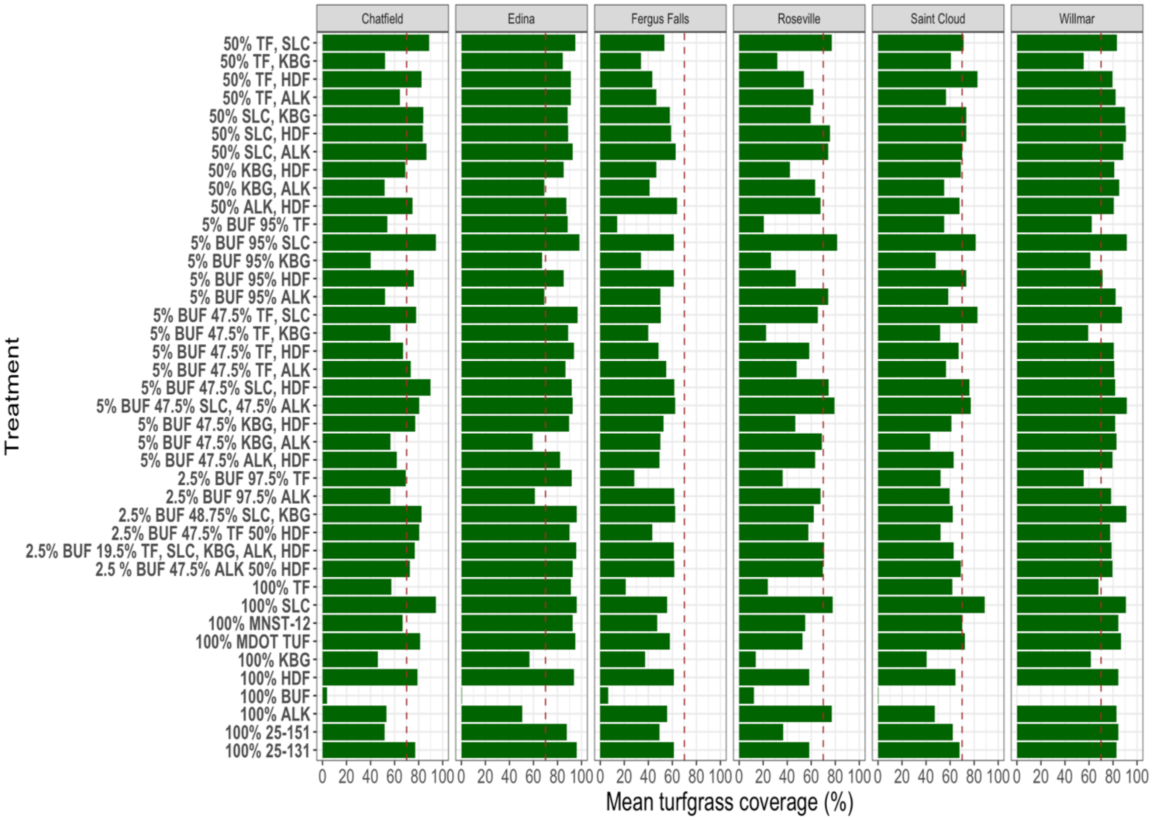

Figure 4.14: Turfgrass coverage sampling in the first fall (1) and spring (2) for the seeding cluster located in southwest Minnesota. Marshall was sampled in fall 2018 (1) and spring 2019 (2). Worthington was sampled in fall 2019 (1) and in spring 2020 (2). Marshall experienced less growing degree days (483 and 79 base 0 ºC and 10 ºC, respectively) than Worthington, which received the second most of all research sites (733 and 241 base 0 ºC and 10 ºC, respectively), prior to winter (Table 4.5). TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This cluster was plotted separately due to plot size limitations.

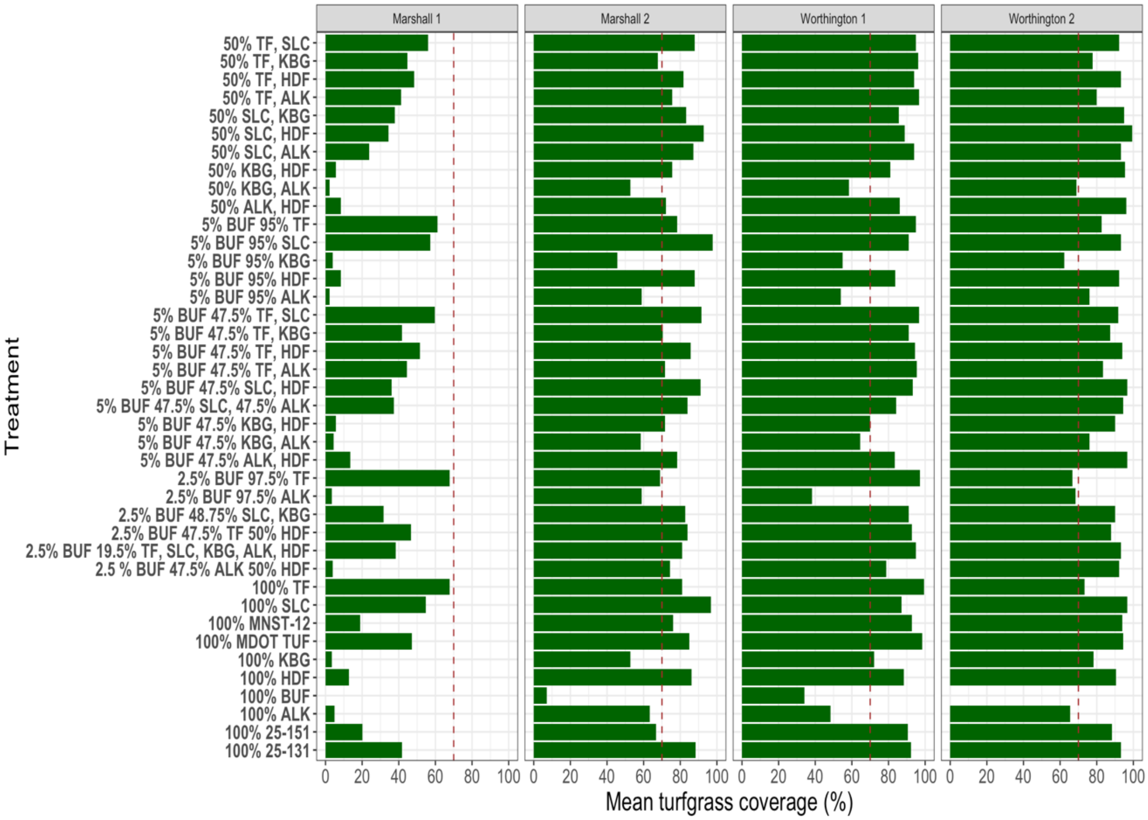

Figure 4.15: Turfgrass species composition coverage sampling for sites classified within the poor soil quality cluster sampled in the fall approximately one year after seeding. Grand Rapids and Brainerd were sampled in fall 2019, and Bemidji and Duluth were sampled in fall 2020. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

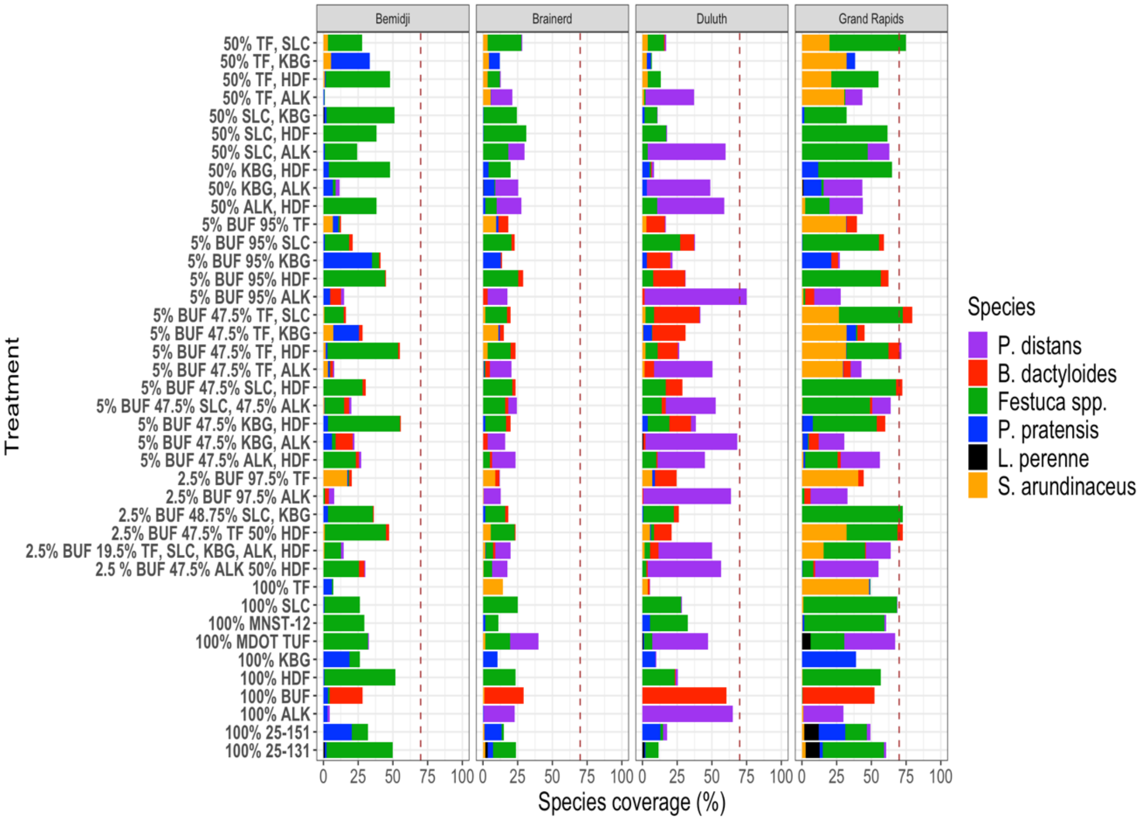

Figure 4.16: Turfgrass species composition coverage sampling for sites classified within the poor soil quality cluster sampled in the spring (4) and fall (5) approximately one and a half and two years after seeding, respectively. Grand Rapids and Brainerd were sampled in spring 2020 (4) and fall 2020 (5), and Bemidji and Duluth were sampled in spring 2021 (4). TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

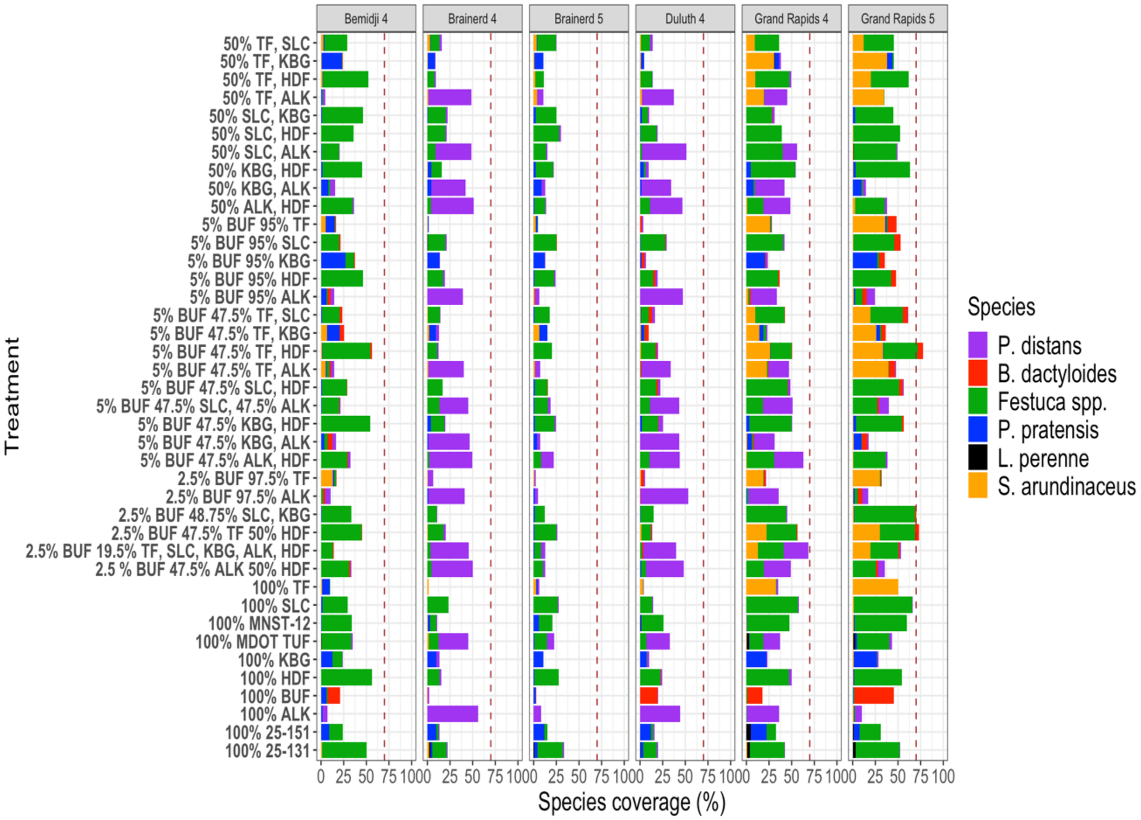

Figure 4.17: Turfgrass species composition sampling for sites within the northern geographical cluster. The third sample time was in the fall approximately one-year after seeding. The fourth was in the spring approximately one and a half years after seeding. The fifth sampling time corresponded to approximately two-years after seeding. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

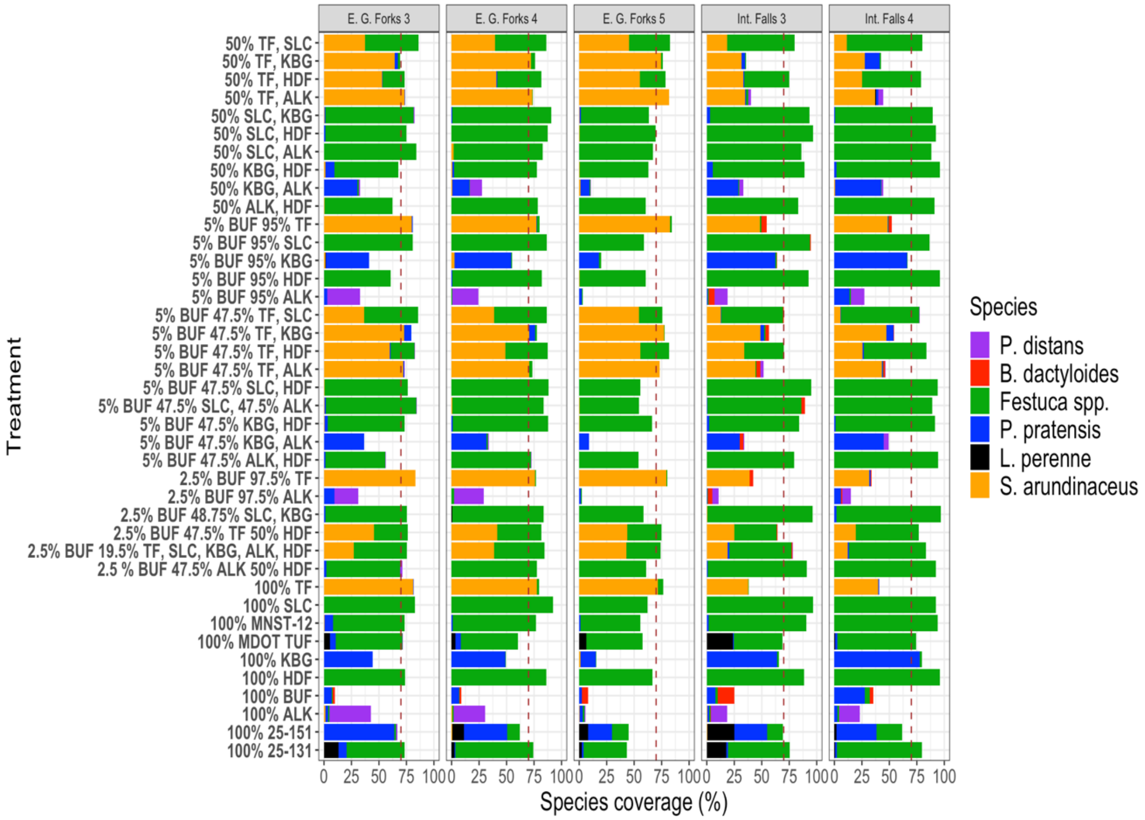

Figure 4.18: Turfgrass species composition coverage sampling at the third sampling time, approximately one-year after seeding. These sites are contained within the seeding cluster located in central to southeast Minnesota. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

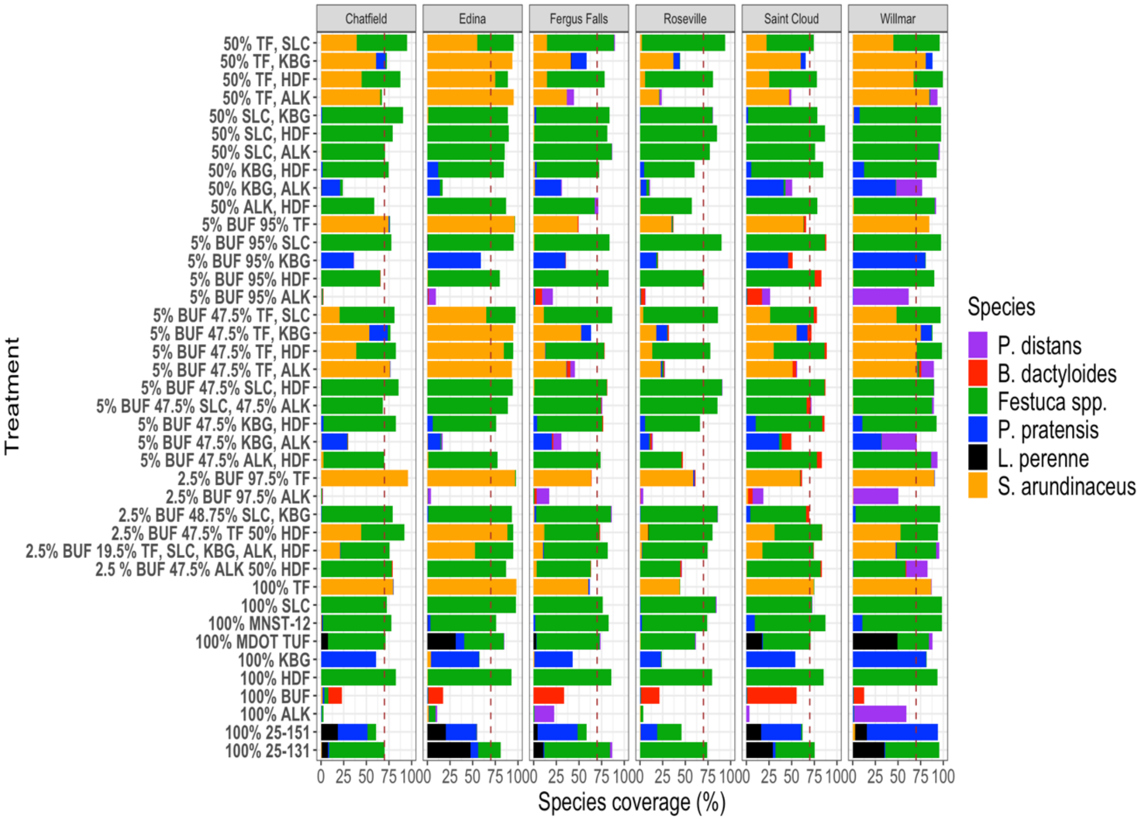

Figure 4.19: Turfgrass species composition coverage sampling at the fourth sampling time at the seeding cluster with sites located in central to southeast Minnesota. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

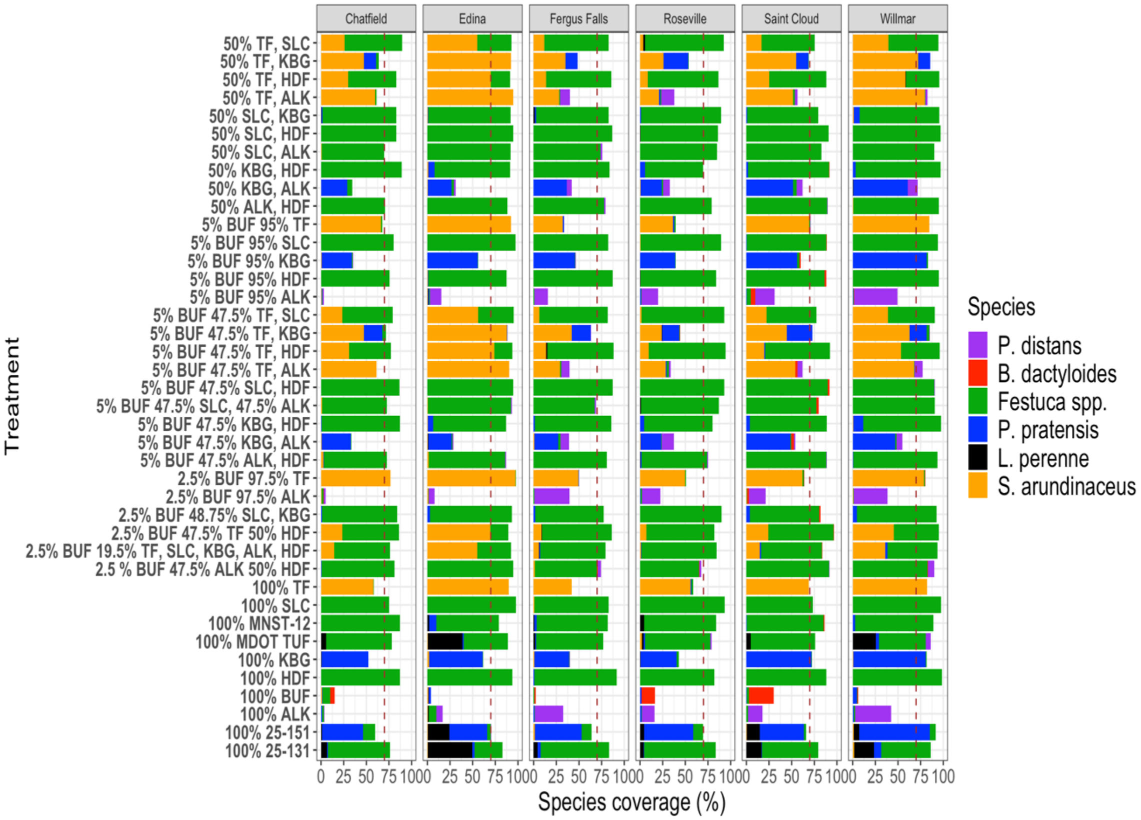

Figure 4.20: Turfgrass species composition coverage sampling at the fifth sample time for the seeding cluster with sites located in central to southeast Minnesota. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss.

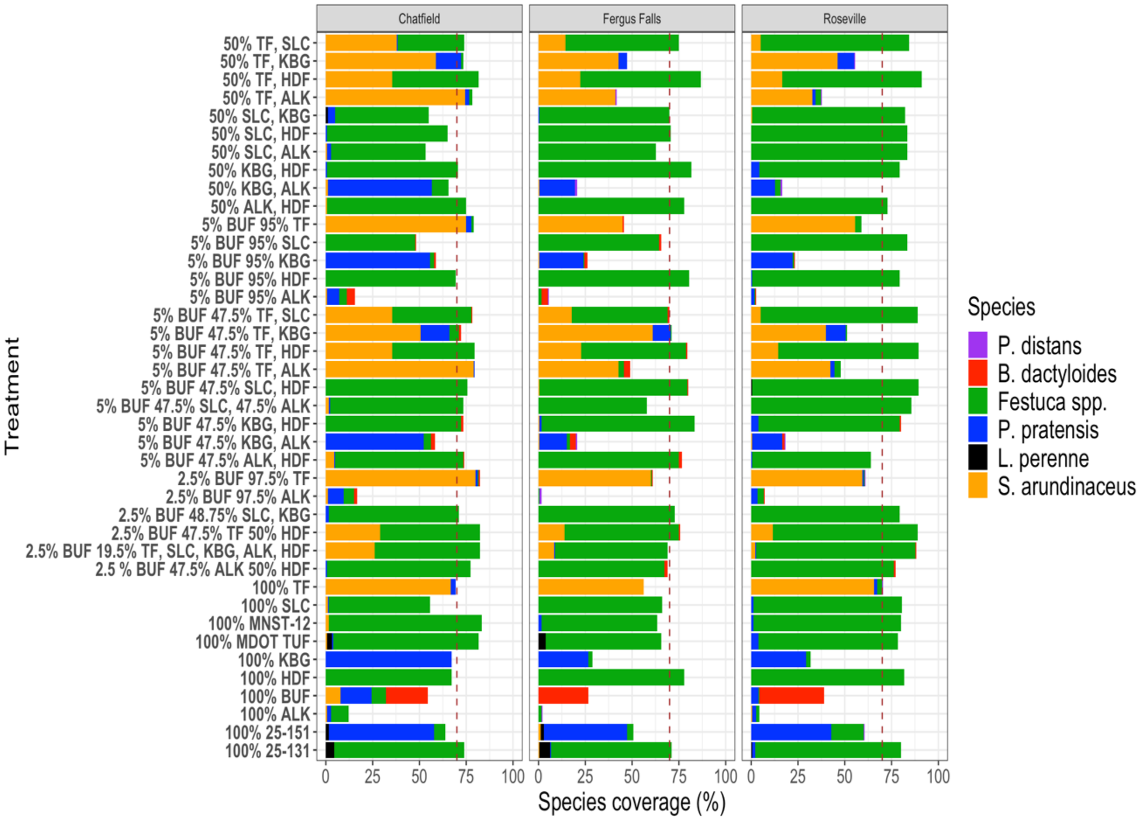

Figure 4.21: Turfgrass species composition coverage for the seeding cluster located in southwest Minnesota. Sampling shows the third, fourth, and fifth sampling dates. The third sample time was in the fall approximately one-year after seeding. The fourth was in the spring approximately one and a half years after seeding. The fifth sampling time corresponded to approximately two-years after seeding. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This cluster was plotted separately due to plot size limitations.

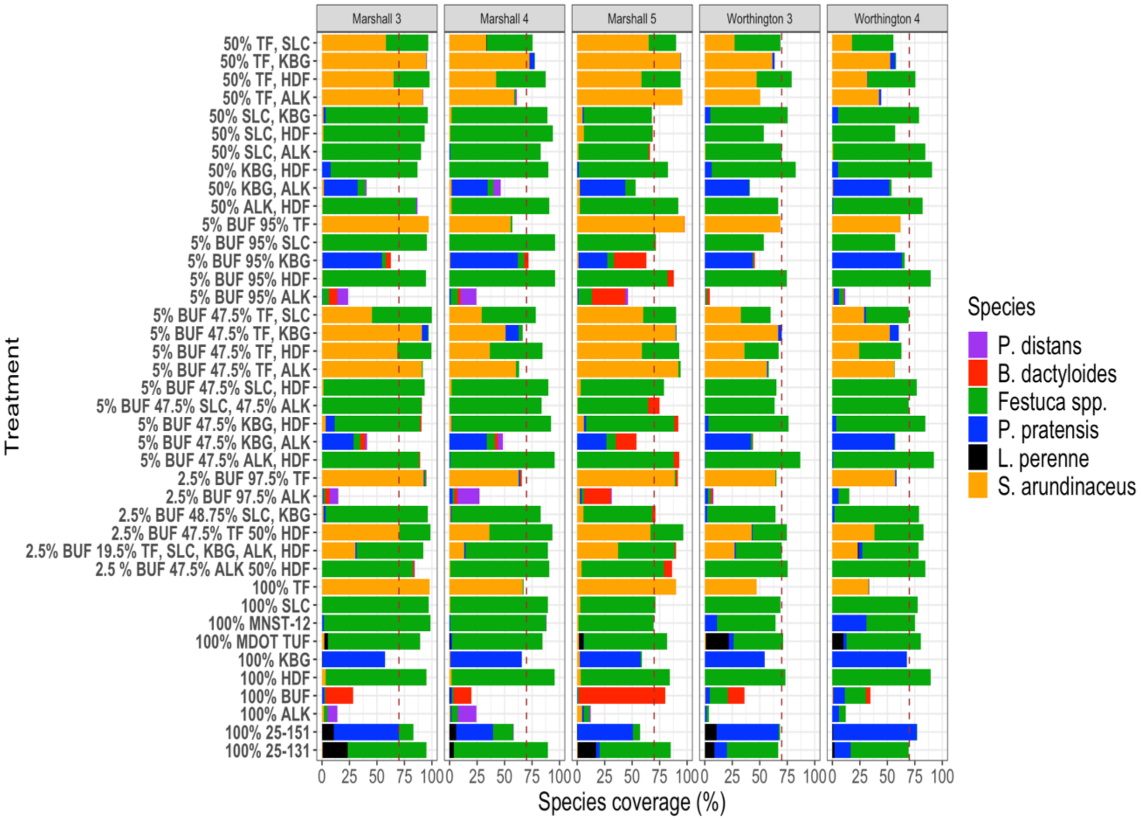

Figure 4.22: Average turfgrass coverage for the three clusters in the first fall sampling time before winter. Northern sites are represented by East Grand Forks and International Falls. Poor soil quality sites contain: Bemidji, Brainerd, Duluth, and Grand Rapids. Central/southern sites include Chatfield, Edina, Fergus Falls, Marshall, Roseville, St. Cloud, Willmar, and Worthington. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This figure is intended for a quick comparison between the three clusters since its biological interpretation is reduced due to averaging.

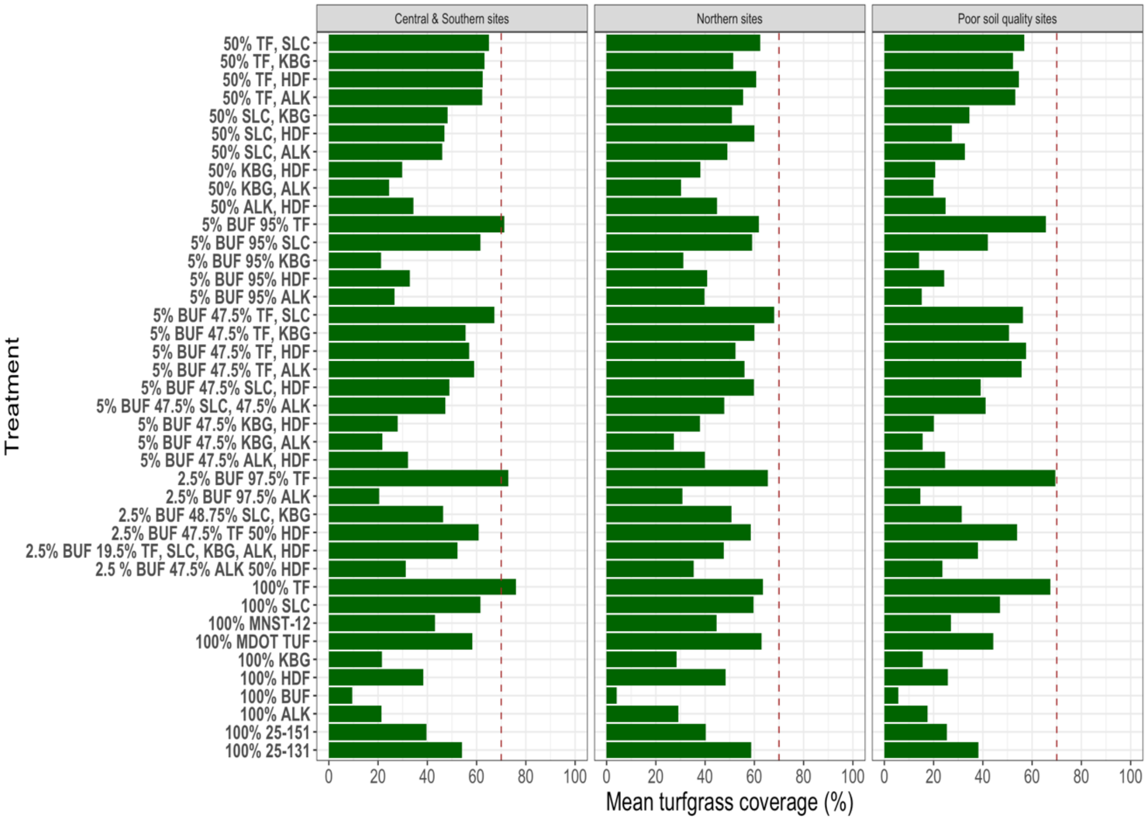

Figure 4.23: Average turfgrass coverage for the three clusters in the second sampling time after the first winter. Northern sites are represented by East Grand Forks and International Falls. Poor soil quality sites contain: Bemidji, Brainerd, Duluth, and Grand Rapids. Central/southern sites include Chatfield, Edina, Fergus Falls, Marshall, Roseville, St. Cloud, Willmar, and Worthington. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This figure is intended for a quick comparison between the three clusters since its biological interpretation is reduced due to averaging.

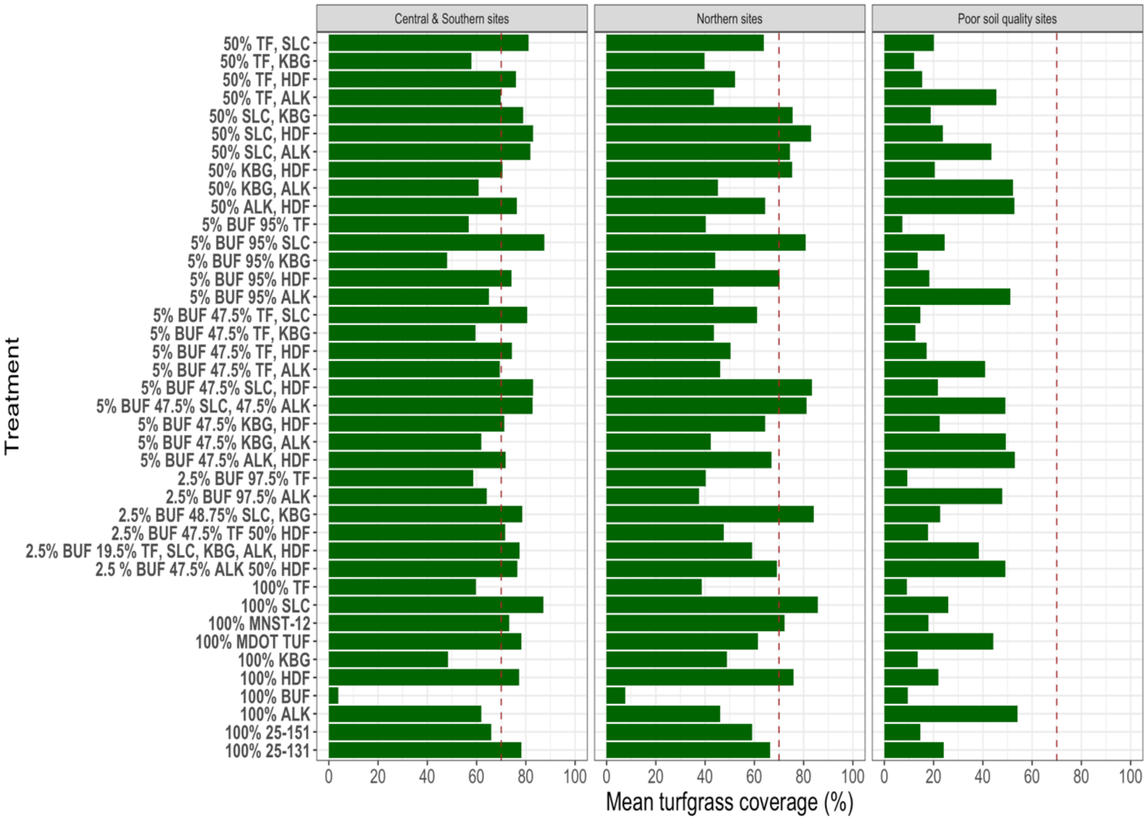

Figure 4.24: Average species composition for the three clusters in the third sampling time approximately one year after seeding. Northern sites are represented by East Grand Forks and International Falls. Poor soil quality sites contain: Bemidji, Brainerd, Duluth, and Grand Rapids. Central/southern sites include Chatfield, Edina, Fergus Falls, Marshall, Roseville, St. Cloud, Willmar, and Worthington. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This figure is intended for a quick comparison between the three clusters since its biological interpretation is reduced due to averaging.

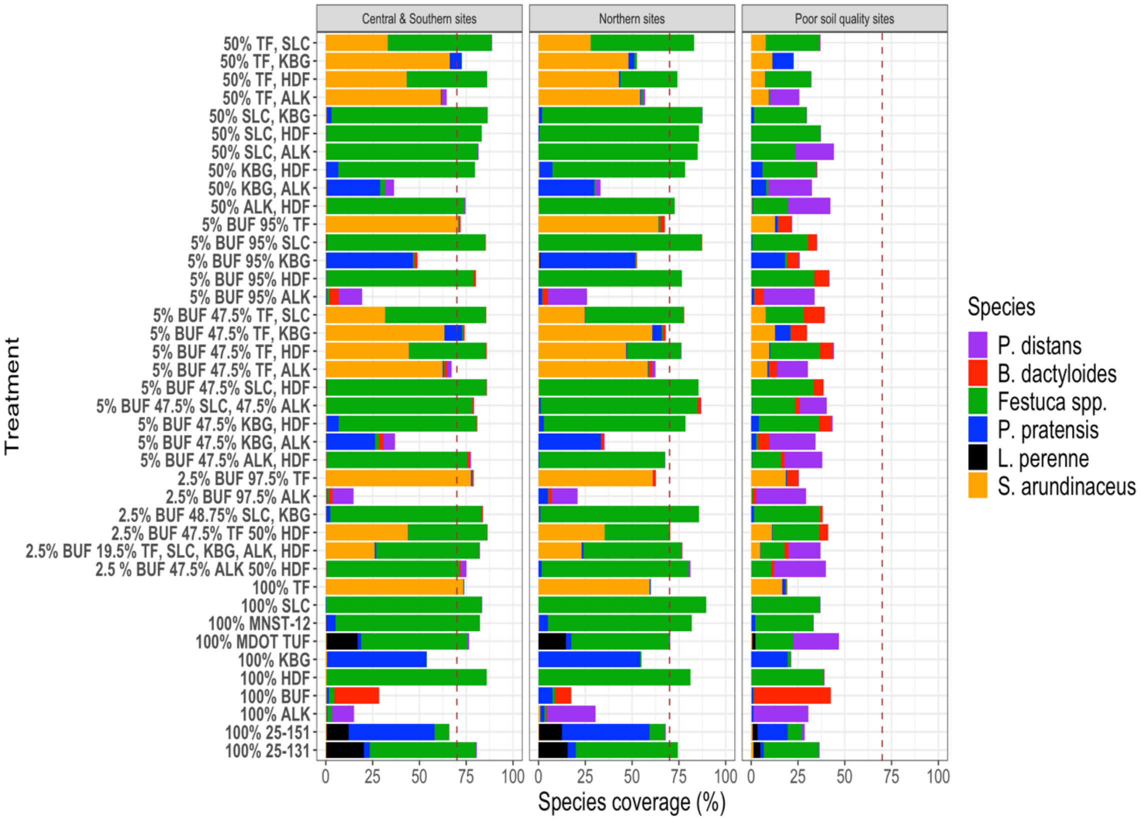

Figure 4.25: Average species composition for the three clusters in the fourth sampling time approximately one and half years after seeding. Northern sites are represented by East Grand Forks and International Falls. Poor soil quality sites contain: Bemidji, Brainerd, Duluth, and Grand Rapids. Central/southern sites include Chatfield, Edina, Fergus Falls, Marshall, Roseville, St. Cloud, Willmar, and Worthington. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This figure is intended for a quick comparison between the three clusters since its biological interpretation is reduced due to averaging.

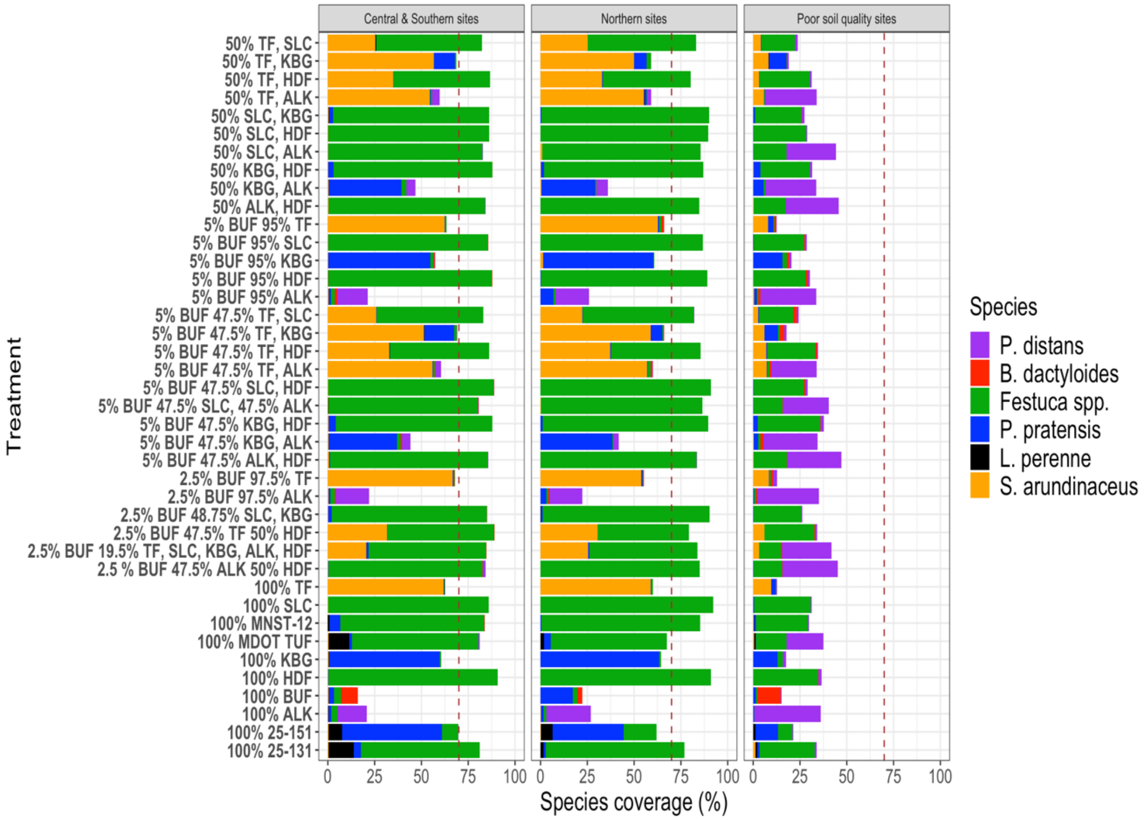

Figure 4.26: Average species composition for the three clusters in the fifth sampling time approximately two years after seeding. Sites seeded in fall 2019 are not included, since data is not available. Northern sites are represented by East Grand Forks. Poor soil quality sites contain Brainerd and Grand Rapids. Central/southern sites include Chatfield, Fergus Falls, Marshall, and Roseville. TF = tall fescue, HDF = hard fescue, SLC = slender creeping red fescue, ALK = alkaligrass, KBG = Kentucky bluegrass, BUF = buffalograss. This figure is intended for a quick comparison between the three clusters since its biological interpretation is reduced due to averaging.

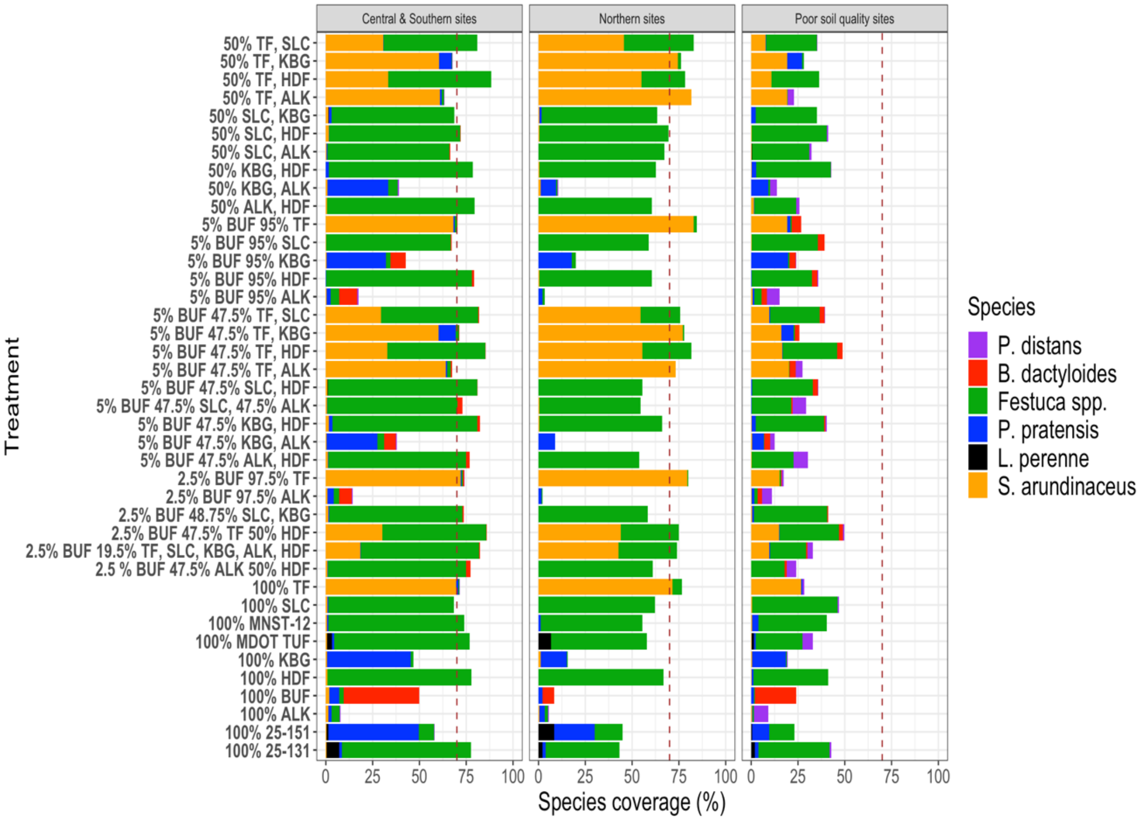

Table 4.9: The average turfgrass species monoculture coverage and standard deviation (shown in parenthesis) at different clusters and across all research sites. Data is from the third sampling time which was collected approximately one year after seeding at each research site. Means comparison show statistical differences among monoculture species within each locality/cluster category. Means comparison are based on Fisher's LSD test with no p-value correction applied.

| | Locality/cluster | | | |
|---|---|---|---|---|
| Monoculture species | Northern | Central/southern | Poor soil quality | All research sites |
| Alkaligrass | 26.1 c (15.5) | 11.8 e (19.9) | 29.4 abc (25) | 18.9 d (22.1) |
| Buffalograss | 8.61 c (11.8) | 23.9 d (17.2) | 41.1 a (18.5) | 26.6 d (19.7) |
| Hard fescue | 81.1 a (10.1) | 85.5 a (9.7) | 38.5 a (23.3) | 71.4 a (25.6) |
| Kentucky bluegrass | 54.2 b (13.4) | 53.1 c (20) | 19.4 bc (15.5) | 43.7 c (23.5) |
| Slender c. red fescue | 89.7 a (9) | 83.3 ab (17) | 36.4 ab (26.7) | 70.8 a (29.2) |
| Tall fescue | 59.4 b (26.4) | 73.5 b (21.9) | 16.7 c (21.8) | 55.3 b (33.4) |
| Average[a] | 53.2 a (32.3) | 55.2 a (33.7) | 30.3 b (23.3) | 47.8 (32.7) |

[a] Statistical means comparing average among clusters.

# Chapter 5

# Conclusion and Final Recommendations

## Summary of findings

Our first objective was to identify and characterize the seed bank vegetation that exists at 14 roadside research sites in Minnesota. We also sought to understand if characteristics of these seed banks affect weed coverage of seeded turfgrass stands. At different roadside research sites across Minnesota, we found differences in seedling density (23-209 seedlings $L^{-1}$), observed species density (8-16 species $L^{-1}$), and found more non-native to native emerged seedlings as a proportion (0.74 to 0.17), than observed species richness (0.48 to 0.41). Even though the seed bank was different across sites, it had little impact on the amount of weed coverage over time. The results suggest that it is more important to maintain seeded turfgrass coverage to reduce weed abundance along roadsides.

Our second objective tested the effects of more seeded turfgrass species diversity on coverage over time at these same roadside research sites. All roadside research sites were adjacent to two to four lane roadsides in Minnesota. Our results showed a significant positive effect of the interaction between time, defined as the order of sampling instances, and turfgrass diversity ($p<0.001$). This means that increases in turfgrass species richness from 1 to 6 species resulted in greater turfgrass coverage, and that this relationship increased through time. The time and number of turfgrass species interaction was not significant for the reduction of weed coverage, but when this interaction was not included, we found a highly significant ($Est=-0.55$, $SE=0.05$, $p<2x10^{-16}$) main effect for a negative relationship between turfgrass species richness and weed coverage. Our results concluded that enhancing seeded turfgrass diversity when developing a seed mixture results in improved turfgrass coverage persistence over time across 14 roadsides in Minnesota.

Our third objective was to identify if different turfgrass seeding clusters incorporating both weather and soil variables in Minnesota would improve upon current recommendations. Turfgrass mixtures determined by soil and weather factors of the seeded site could allow more adapted species and ratios throughout Minnesota. We found differences in soil chemical and physical characteristics and observed weather among sites. We used an agglomerative hierarchical cluster analysis and identified different cluster solutions between the 14 research sites. We validated our cluster results based on closely examining our turfgrass monoculture coverage and standard deviation, and we identified, two geographical roadside turfgrass seeding clusters for Minnesota (northern, central/southern) and one non-geographical cluster for sites with poor soil quality (low organic matter, greater sand content in soil texture, higher bulk density, and greater saturated paste extract electrical conductivity). Recommending these clusters for Minnesota roadsides seeded to turfgrass can continue to improve the establishment and persistence of roadside vegetation.

## Recommendations

From our findings we qualitatively developed the following roadside turfgrass seed mixtures for the state of Minnesota (Table 5.1). These mixtures are recommended in addition to the currently recommended mixtures. We limited the species recommendations to ones that were directly tested and evaluated in this experiment. All mixtures were designed based on pure live seed ratio and then the relative weight was calculated from that ratio for each constituent species. In discussing all seed mixtures below, we refer to seed ratio, unless noted by weight.

We recognize there are some limitations in the regionality of our clusters in Minnesota. Consider Duluth, Minnesota which is closer to Lake Superior and is in a different plant hardiness zone than areas located further inland (*USDA Plant Hardiness Zone Map*, 2012). Additionally, we recognize that southeast and southwest Minnesota can be distinguished by a precipitation gradient, but both are grouped within the central/southern cluster. These areas of the state could be later delineated. It is important to remember that clustering is a simplification process explaining a portion, and not of all the variability along roadsides tested in this experiment. The plant hardiness zones are also continuing to change with climate in Minnesota. Future testing and validation of these clusters in Minnesota will be needed over time, since species' zones of adaptation will change (McKenney et al., 2007).

We want to stress the importance of including multiple species in a seed mixture. If the area and time are expanded for the use of these mixtures, then what appears to be an overly complicated mixture for a single site, may be a more appropriate one. Larger areas have greater nuances in sunlight quality and quantity, soil chemical and physical characteristics, weather and climate, maintenance, and other disturbance factors. These mixtures recommendations are effectively zooming out to answer questions over larger areas and longer periods of time.

### Northern cluster

The northern mixture was designed similarly to a Michigan state department of transportation mixture (MDOT TUF), which has previously performed well in several states and at harsh sites (Watkins et al., 2019). Our northern mixture differed from MDOT TUF to include no perennial ryegrass, slightly more weeping alkaligrass (from 16.30% to 20%), and we added tall fescue (5%), due to some adaptation of this species at the East Grand Forks research site in northwest Minnesota. Tall fescue was included at a low rate since it germinates quickly and has the potential to overwhelm a mixture in the seedling stage. We also approximately flipped the ratio of *F. ovina*: *F. rubra* in this mixture compared to the Michigan mixture, since hard fescue showed similar performance to slender creeping red fescue at sites located in this region.

### Central/southern cluster

The central/southern regional turfgrass mixtures included more tall fescue (+5%) and less weeping alkaligrass (-10%) than the northern mixture. The central/southern mixture differed from MNST-12 turfgrass seed mixture (Minnesota Crop Improvement Association, 2021) by including tall fescue (from 0 to 10%), and weeping alkaligrass (from 0 to 10%), and a higher proportion of *F. ovina* compared to the *F. rubra* complex

(2:1 *F. ovina* to *F. rubra* for the central/southern cluster mixture compared to 0.25-1:1 by weight in the MNST-12 mixture). Tall fescue was included at a slightly greater ratio in the central/southern cluster due to it being more adapted to the warmer temperatures in this area of Minnesota. Alkaligrass showed poorer performance in this area of the state, so its ratio was lowered. Alkaligrass was likely less tolerant of the warmer drier conditions, although some of those conditions existed at the Duluth site and alkaligrass is persisting. The ratio of fine fescue species was modified since 'Gladiator' hard fescue has shown less stress in the middle of the growing season in this region and contains a lower standard deviation than 'SeaMist' slender creeping red fescue. The persistence of hard fescue in Minnesota along roadsides has been identified previously by Friell et al. (2015).

### Poor soil quality cluster

The poor soil quality cluster contained more alkaligrass than the northern cluster (+10%). A significant portion of weeping alkaligrass was included in the mixture, since in roadsides that are heavily salted and containing poor soils, it can be the only species to survive in an experiment (Watkins et al., 2019). In our study, alkaligrass did well at three of the four research sites clustered within the poor soil quality cluster, but still tended to decrease over time. Tall fescue was not included in the mixture, since it was nearly absent at three of the four research sites, and has previously shown susceptibility to winter stresses along roadsides in Minnesota (Friell et al., 2015). The conditions at these sites seem to be exacerbating the death of tall fescue. Kentucky bluegrass was included at a low rate in this mixture (5%), since its performance is limited, but it may provide some benefit in certain roadside situations of this type. Hard and slender creeping red fescue were included at a similar rate, since slender creeping red fescue has shown greater salt tolerance in these conditions. Buffalograss was included at 5%, since it has natural adaptations to moisture and temperature extremes and its performance at the Duluth site shows that it can survive difficult winter conditions. Buffalograss' performance in excessively sandy soils can be limited based on our results from Brainerd, although Severmutlu et al. (2011) found it may not be a significant factor for turf-type cultivars in a warm-climate. Additional research to identify and select for buffalograss cultivars that are better adapted to roadsides would be beneficial.

To identify a poor soil quality site, we recommend more soil testing before seeding. We define a poor soil quality site as meeting two of the three following criteria: soil sand content > 55%, organic matter <= 2.2% (Hopkinson et al., 2016), and bulk density >=1.6 g $cm^{-3}$, or if the site meets one of the three following criteria: soil sand content > 70%, organic matter <= 1.7% (Hopkinson et al., 2016), or bulk density >= 1.8 g $cm^{-3}$. There are also more species potentially adapted to this cluster that were not tested in our experiment, so this mixture would benefit from additional research. A brief discussion of other species adapted to this cluster is included below.

### Other considerations and species with potential for roadsides

Perennial ryegrass was not recommended to remove risk of compromising a mixture. For example, even if the seed ratio was designed properly, if the total seeding rate was doubled, then perennial ryegrass' seeding density is effectively doubled for that given area, and this has the potential to limit other species in a mixture (Dunn et al., 2002; Engel & Trout, 1980; Henensal et al., 1980). Previous MnDOT recommendations have included a blend of Kentucky bluegrass within a mixture for roadsides (MnDOT, 2014), and we would continue to encourage this practice, since it does offer benefits in seed mixture diversity. Although, preference should be given to compatible species diversity before additional cultivar diversity. We also know that older varieties of Kentucky bluegrass are generally performing better on roadsides (Friell & Watkins, 2020), so when selecting appropriate cultivars older ones should not be overlooked.

There are various reasons why species have been historically excluded from roadside turfgrass mixtures. Sand lovegrass [*Eragrostis trichodes* (Nutt.) A. W. Wood] and switchgrass (*Panicum virgatum* L.) are excluded due to their tall stature. Squirrel-tail barley grass (*Hordeum jubatum* L.) is a noxious-like weed due to its long awns. Tall fescue should be excluded at sites with poor soil quality with current cultivars since it does not persist. Redtop bentgrass and other species from the *Agrostis* genus have the potential to be invasive, although upland bentgrass [*Agrostis perennans* (Walter) Tuck.] is worth investigating (Engelhardt & Ratliff, 2019). Smooth bromegrass was not included in turfgrass mixtures due to its poorer adaptation to regular mowing (White & Smithberg, 1972) and its ability to be invasive, since this species is highly abundant on roadsides in Minnesota (Biesboer et al., 1998). Smooth bromegrass also has mixed performance in roadside experiments at this time (Duell & Schmit, 1975; Watkins et al., 2019). A number of currently available cultivars of grasses may not have adequate salt or winter tolerance at this time, such as timothy grass, blue grama, sideoats grama, and prairie junegrass (Friell et al., 2013; Stenlund & Jacobson, 1994), but there may be possible benefits to the addition of such species along roadsides.

There are additional species that could be further investigated in roadside turfgrass mixtures. These species could be more applicable for the poor soil quality cluster and have shown some potential in historical research. Western wheatgrass has shown compatibility with low-maintenance turfgrass species (Bunderson, 2007; Robins & Bushman, 2020) and its natural drought tolerance could yield it to be a good component for sandy roadsides. Canada bluegrass, Russian wildrye, and sand dropseed have been observed to perform well on excessively sandy roadside sites in Minnesota (Foote et al., 1978; Henslin, 1982). Poverty dropseed was found in the soil seed bank at 10 of 14 roadside research sites and has been observed growing along many roadsides in Minnesota. Purple lovegrass has been observed growing at the Bemidji roadside research site which is excessively sandy. White clover was included in historical MnDOT mixtures and it is well adapted to many soil textures (Lane et al., 2019). Common yarrow (*Achillea millefolium*) has been observed growing at the Duluth research site and tolerating droughty conditions at other roadside research sites.

## Limitations

Our study contained limitations that skewed the relative performance of some species. These limitations may not be entirely reflective in the coverage data and could mislead some characterizations of this research. For instance, our sampling timing in the spring occurred when tall fescue was not always actively growing (but still green) and occasionally when buffalograss had not even greened up. Therefore, the spring sampling dates contain some bias of less coverage of these species. This occurred at some fall sampling times as well, such as at Worthington or Marshall when sampling occurred in November, because at this time the coverage of buffalograss was beginning to go dormant and leaf tissue was not as expansive compared to mid-summer. We recommend one mid-summer (June-August) sampling in Minnesota for all future roadside turfgrass experiments, especially if they contain a warm-season species. A summer sampling would also show reductions of slender creeping red fescue at southern Minnesota sites.

The total amount of time we sampled the roadside vegetation is still relatively low compared to some previous regional roadside work in Minnesota. We especially noticed buffalograss and Kentucky bluegrass coverage to be increasing at some sites over time. A length of five years seems more sufficient as a minimum amount of time to evaluate coverage changes, especially in a mixture setting.

## Future research ideas

- Based on the significance of the dendrogram cluster, it would be most beneficial to continue to test and improve the mixture for the poor soil quality cluster along roadsides in Minnesota.
- The ratios and seeding timing of warm and cool-season grass mixtures should be investigated. Specifically, a seedling competition study between buffalograss, hard fescue, and Kentucky bluegrass would be beneficial. Treatments could consist of planting date, mowing height, and different mixture ratios and coverage could be evaluated over time similar to Brede & Duich (1984b). This could improve the recommendation of the poor soil quality cluster mixture (Table 5.1).
- We recommend identifying, improving, and testing germplasm for roadsides. A couple characteristics that could be selected for would be for improved winter hardiness of tall fescue and overwintering of buffalograss on roadsides in Minnesota. Ideally, germplasm would be selected from roadsides in these different clusters or

seed provisional zones (Bower et al., 2014). A continued assessment of adapted and less adapted germplasm seeded along roadsides is necessary.

- An optimum planting date and range for turfgrass mixtures could be calculated based on the ideal or adequate number of growing degree days before winter in both geographical clusters.
- In the future, it will be important to identify if Minnesota continues to trend mostly warmer and wetter (NOAA; MnDNR, State Climatology Office) or if it becomes dryer and warmer, and the relative deviation in these trends. Based on the current changes and trajectory in climate, turfgrass mixtures for roadsides should be open to future modification.
- We encourage testing and refinement of these seeding cluster for roadside turfgrass mixtures in Minnesota. Cluster and species refinement could consist of modification, addition, or subtraction of regions. Species modification could consist of adding or removing species, modifying species ratios, or the addition or subtraction of cultivar diversity. All modifications should be tested and recommended by pure live seed ratio.

Table 5.1: Recommended turfgrass seed mixtures for different seeding clusters in the state of Minnesota. PLS = pure live seed, PLW = pure live weight.

| Seeding cluster[a] | Species type | Scientific name | Common name | PLS (%) | PLW (%)[c] |
|---|---|---|---|---|---|
| North | Cool season | *Puccinellia distans* | Weeping alkaligrass | 0.20 | 0.07 |
| North | Cool season | *Poa pratensis* | Kentucky bluegrass [b] | 0.20 | 0.10 |
| North | Cool season | *Schedonorus arundinaceus* | Tall fescue | 0.05 | 0.13 |
| North | Cool season | *Festuca brevipila* | Hard fescue | 0.35 | 0.41 |
| North | Cool season | *Festuca rubra* ssp. *littoralis* | Slender creeping red fescue | 0.20 | 0.30 |
| Central/southern | Cool season | *Puccinellia distans* | Weeping alkaligrass | 0.10 | 0.03 |
| Central/southern | Cool season | *Poa pratensis* | Kentucky bluegrass [b] | 0.20 | 0.08 |
| Central/southern | Cool season | *Schedonorus arundinaceus* | Tall fescue | 0.10 | 0.23 |
| Central/southern | Cool season | *Festuca brevipila* | Hard fescue | 0.40 | 0.40 |
| Central/southern | Cool season | *Festuca rubra* ssp. *littoralis* | Slender creeping red fescue | 0.20 | 0.26 |
| Poor soil quality | Cool season | *Puccinellia distans* | Weeping alkaligrass | 0.30 | 0.06 |
| Poor soil quality | Cool season | *Poa pratensis* | Kentucky bluegrass [b] | 0.05 | 0.01 |
| Poor soil quality | Warm season | *Buchloe dactyloides* | Buffalograss | 0.05 | 0.47 |
| Poor soil quality | Cool season | *Festuca brevipila* | Hard fescue | 0.30 | 0.20 |
| Poor soil quality | Cool season | *Festuca rubra* ssp. *littoralis* | Slender creeping red fescue | 0.30 | 0.26 |

[a] Additional research is recommended to clarify the design of the seed mixture for the poor soil quality cluster, since this mixture is based only on what species we tested, and from historical roadside literature and personal field observations, other species may also be applicable and beneficial.

[b] Kentucky bluegrass seed weight can vary by a factor of almost three times depending on the cultivar and seed lot (Christians et al., 1979).

[c] Weight ratios were calculated by collecting standard seed weight from my calculations and other sources (Beard, 1973; Engelhardt, 2016; Hollman et al., 2018; USDA plant fact sheet).

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