HortScience 54(5):824–828. 2019. https://doi.org/10.21273/HORTSCI13779-18

Turfgrass Species Affect the Establishment and Bloom of Kura Clover (*Trifolium ambiguum*) in Lawns

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Additional index words. Kura clover, urban diversity, reconciliation ecology, flowering lawns

***Abstract*. Lawns represent one of the largest cultivated areas in urban landscapes, and in the Upper Midwest of the United States, lawns are typically composed of a small number of cool-season turfgrass species. There is increased interest in enhancing areas dedicated to lawns using flowering species for conservation purposes—for example, to support pollinators. In this study we used a model flowering forb, Kura clover (*Trifolium ambiguum* M. Bieb.), because—like many flowering species of conservation interest—it is slow to establish and is sensitive to grass competition. We varied the Kura clover seeding rate into four different turfgrass species treatments: kentucky bluegrass (*Poa pratensis* L.), hard fescue (*Festuca brevipila* Tracy), tall fescue [*Schedonorus arundinaceum* (Schreb.) Darbysh.], and perennial ryegrass (*Lolium perenne* L.) in two separate trials. Establishment and bloom of Kura clover was significantly greater in trial 1 for kentucky bluegrass and hard fescue than tall fescue and perennial ryegrass. In trial 2, Kura clover established significantly greater in kentucky bluegrass compared with tall fescue and perennial ryegrass, whereas Kura establishment in hard fescue was not significantly different from the other treatments. The seeding rate of Kura clover did not affect establishment in either trial. The results from this study suggest kentucky bluegrass and hard fescue are promising turf companion grasses for future forb/turf interseeding research.**

Turf lawns are estimated to cover roughly 2% of the continental United States (Milesi et al., 2005), mostly in highly urban areas. Although management of lawns varies by function and individual manager, it is typically characterized by three primary cultural practices: mowing, fertilizing, and irrigation (Turgeon, 1999). These practices are intended to favor turf species and, when applied in tandem with proper establishment techniques, result in stands of uniform turf. Despite lawns being currently managed as uniform monocultures, they are often host to flowering plants that provide foraging resources for bees and other pollinators. A recent insect survey of park lawns hosting dandelion (*Taraxacum officinale* F.H. Wigg.) and white clover (*Trifolium repens* L.) conducted in Lexington, Kentucky (Larson et al., 2014), found 37 associated bee species. These plant species are typically considered weeds in the United States and are sometimes eliminated through the use of broadleaf herbicides. However, lawns managed intentionally for forb abundance and richness would likely have a beneficial impact to local foraging bee communities. Previous research has suggested a strong positive relationship between forb community richness and pollinator community richness that likely extends to lawn communities as well (Ebeling et al., 2008; Potts et al., 2003). Such management goals would necessitate the reduction of other lawn inputs and would further the goal of increased sustainability. Mowing often correlates negatively with plant species richness in lawns (Bertoncini et al., 2012; Garbuzov et al., 2014; Lerman et al., 2018; Shwartz et al., 2013; Smith and Fellowes, 2015), and managing floral lawns could lead to less-intensive mowing regimes. The inclusion of legumes such as white clover has shown to enhance turf nitrogen uptake through nitrogen fixation (McCurdy et al., 2014; Sincik and Acikgoz, 2007), and would potentially reduce fertilizer inputs into lawns.

At the other extreme of flowering lawns, researchers as the University of Reading in the United Kingdom have abandoned the use of turfgrasses altogether, and have been developing species lists and management practice guidelines for purely floral lawns (Smith and Fellowes, 2015) that have been found to have benefits to flower-visiting insects (Smith et al., 2014). These lawns may provide benefits to flower-visiting insects, but they are not meant to be areas of high human traffic and recreation. Human traffic on monocultures of white clover was found to reduce green cover up to 14 times faster when compared with hybrid bermudagrass [*Cynodon transvaalensis* Burtt-Davy 3 *C. dactylon* (L.) Pers.], suggesting the importance in turfgrasses for maintaining long-term cover in variable traffic conditions (Brosnan et al., 2014). Another potential challenge of these nonturfgrass plantings is the reliance on precultivation and installation, which can be costly on a large scale, and the potential need for more specialized care. In addition, there is still a strong cultural connection with turf lawns (Harris et al., 2013), and an intermediate flowering lawn that combines turfgrass and floral species may have broader and more practical appeal, especially for recreational use. For these reasons, grass–forb mixes established from seed are an important area of focus for future research. This type of floral-enhanced lawn should provide quick groundcover, reduce lawn maintenance, save money, and provide typical lawn functions such as recreation.

Although floral lawns hold great promise in improving biodiversity in urban areas, guidelines for establishing usable forbs in a lawn are needed to encourage adoption. The main objective of this study was to identify a turfgrass species that would allow for better success in the introduction of flowers not typically thought of as turf weeds. An ideal turfgrass species would maintain groundcover, but also allow for establishment and bloom of its forb companions. Some effort has been made into developing seeding strategies for white clover into established lawns to benefit pollinators and soil nitrogen (McCurdy et al., 2013; Sparks et al., 2015). White clover is an established agronomic crop that is widely associated with pasture agriculture, and is well adapted to the grazing systems under which it evolved (Leffel and Gibson, 1973). Pasture systems and lawns are similar in many ways, (e.g., compaction and cutting by animals or humans) and it is no surprise that white clover does well in both environments. So although white clover is a viable option for improving lawn floral abundance, more forb options are needed to improve the diversity and conservation value of turfgrass lawns.

To understand more fully how turfgrass species influence other forbs, we selected four common turfgrass species used in home lawns in the northern United States to mix with a species of clover: Kura clover

Received for publication 3 Dec. 2018. Accepted for publication 6 Mar. 2019.
We acknowledge the Environmental and Natural Resources Trust Fund of Minnesota for providing the funding that made this study possible. We also thank Andrew Hollman for his assistance in establishing and maintaining the research trials. Last, we thank Johnathon Tetlie, Jason Ostergaard, and Garett Heineck for their assistance in collecting data.
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(*Trifolium ambiguum* M. Bieb.). Kura clover is a rhizomatous perennial plant originating from eastern Europe/western Asia and has been investigated as a cold-tolerant forage plant for pasture cattle in the United States. Kura clover is not commonly cultivated across the United States, but it can be found growing infrequently in seminatural areas. Kura clover is known for its slow establishment period and sensitivity to grass competition (Hill and Mulcahy, 1995; Seguin et al., 1999) when used under pasture conditions. Kura clover's agronomic properties, such as high seed germination rates and grazing tolerance, combined with its sensitivity to competition make it an ideal model species for isolating how competitive pressure from different turf species might affect the establishment and bloom of flowering plants not typically associated with turfgrass lawns.

We predicted that slow-establishing and nonrhizomatous turf species would favor Kura clover establishment and bloom, whereas fast-establishing and rhizomatous species would disfavor Kura establishment and bloom. Our primary goal was to identify turfgrass species that can be seeded at their recommended rates while minimizing turfgrass species-specific competitive effects. Identifying turfgrass species more amenable to forb species additions is important to subsequent studies aimed at identifying other limiting factors in forb establishment, such as germination and mowing tolerance. This is the first of a series of studies that aims to provide foraging resources for pollinating insects in turf lawns.

Materials and Methods

Species selection. We selected four commonly used cool-season turfgrasses: 'Moonlight SLT' kentucky bluegrass (*Poa pratensis* L.), 'Beacon' hard fescue (*Festuca brevipila* Tracy), 'Grande II' tall fescue [*Schedonorus arundinaceum* (Schreb.) Darbysh.], and 'Apple GL' perennial ryegrass (*Lolium perenne* L.). Hard fescue is a slow-establishing turfgrass species with a bunch-type growth habit. Of the four species selected, we predicted Kura clover to establish and bloom at its highest rate in hard fescue stands. Kentucky bluegrass is a slow-establishing turfgrass species with a rhizomatous growth habit that we predicted to have intermediate levels of Kura clover establishment and bloom. Both perennial ryegrass and tall fescue are commonly known for their ability to establish from seed rapidly, and we predicted the lowest establishment and bloom levels in plots established with these species.

Although seeding guidelines do exist for Kura clover establishment in grass pasture systems (Hill and Mulcahy, 1995; Vandevender, 2003), we chose to manipulate seeding rate because turfgrass systems may change stand quality dynamics. We chose to investigate both the lowest and highest recommendations for seeding: 0.57 and 1.1 $g \cdot m^{-2}$, respectively; as well as an intermediate rate of 0.85 $g \cdot m^{-2}$.

Experimental design and establishment. This study was conducted at the Turfgrass Research, Outreach, and Education Center (TROE) on the University of Minnesota St. Paul campus (lat. 44.9944, long. 93.1849). The soil at the TROE center is dominated by a Waukegan silt loam, which is a deep, well-drained soil with a fine silty top layer over a sandy bottom layer. Two trials were conducted, with the first starting in 2013 and the second in 2014. Both trials were in separate but adjacent plots. Aggregate soil samples were collected at the conclusion of the trials to characterize the soil environment through standard soil testing at the University of Minnesota Soil Testing Laboratory. The soil measurements for phosphorous, potassium, and soil organic matter were nearly identical in the two trials, with the exception of pH (trial 1 pH, 6.5; trial 2 pH, 6.1). Exact soil nutrient and organic matter values can be found in Supplemental Table 1. Trials were organized as a complete random factorial design, with four grass species and three Kura clover seeding rates as experimental factors. Each grass–seeding rate combination was replicated three times for a total of 36 observational units per trial, and combinations were arranged randomly into a six-plot × six-plot grid. Individual plot dimensions were 1 × 1.5 m, and each plot was separated by a 0.15-m border.

The first trial was seeded on 20 June 2013 and the second on 2 July 2014. Temperatures during the first month of establishment averaged a high of 29.5 °C and a low of 20.2 °C for trial 1, and a high of 27.2 °C and a low of 16.3 °C during the first month of trial 2. Sites were prepared by applying glyphosate to clear undesirable plants, rototilling to break up rooting structures, and power raking directly before seeding. Grass species were seeded at rates recommended by Turgeon (1999) (Table 1). Kura clover seeding rate treatments were 0.57, 0.85, and 1.1 $g \cdot m^{-2}$. Grass seed and Kura clover were both hand-seeded concurrently but from separate packets, with short barriers placed around the plot perimeter to prevent seeds from being blown outside the plot area. Because of the variable nature of Kura rhizobium nodulation (Beauregard et al., 2003; Laberge et al., 2005; Seguin et al., 2001), we made the decision not to inoculate at the time of seeding; in addition, we were using Kura as a model for other flowering plants and were not interested in highly successful Kura establishment. Rather, we wanted to observe how the species was affected by competition from common turfgrass species.

Starter fertilizer is a common recommendation for the establishment of new lawns, thus the trial area was given an application of EC Grow Greens Grade Fertilizer (EC grow Inc., Eau Claire, WN) (analysis 10N–7.9P–18.3K) directly after seeding, broadcast-applied at a rate of 3 g N/m^2. The study area was then covered with biodegradable Futerra® EnviroNet (PROFILE products LLC, Buffalo Grove, IL) blankets to prevent seed movement between plots; this type of blanket is sometimes used for lawn establishment, especially on sloped sites to prevent soil erosion. Plots were irrigated twice daily for 20 min during the first 2 weeks to assist establishment. Plots were mowed adhering to the one-third rule, mowing to an 8-cm height if any plot was at least 11 cm in height. Grass clippings were left onsite. Mowing practice and the nonremoval of grass clippings were meant to simulate best management recommendations for low-input turf lawns as provided by the University of Minnesota Extension Service. Data collection began in June of the year following seeding.

Data collection and analysis. Data were collected once per month for 3 months starting in June for each trial, and always preceded a mowing event to avoid introducing a cutting bias on plant metrics. A 1 × 1.5-m quadrat with nylon wire grid lines was used to help break plots visually into 117 12-cm^2 sections to facilitate the counting of Kura clover trifoliate leaves and blooms, which were summed over the entire plot, thereby providing an absolute abundance of trifoliate leaves and blooms. To estimate the percentage of total plot area covered by Kura clover leaves (leaf cover), recorders visualized the number of grid squares covered by Kura leaves as if in one contiguous patch and then divided that number by 117 (the total number of squares in the quadrat). To help control for the qualitative nature of this cover estimate, the leaf cover for a given plot was estimated by two observers and then averaged between them to obtain the final leaf cover number.

Fixed effects used to measure the establishment of Kura clover included trifoliate leaf count, bloom number, and plot leaf coverage. Data were analyzed with the R statistical program (v3.2.3; R core team, Vienna, Austria) using the nlme and multcomp packages. All three fixed effects were first tested for correlation with one another using Pearson's product-moment correlation coefficient. Initial analysis used an analysis of variance of a linear mixed-effects model (except in the case of blooms), in which Kura clover seeding rate and turf species were specified as an interaction term, and trial was an additive term. Because trial was significant in this first model, further analysis focused on each trial individually, keeping turf species and Kura seeding rate as an interaction term. Plot number was used as a

Table 1. List of turfgrass species used and their recommended seeding rate.

Species	Growth habit	Seeding rate ($g \cdot m^{-2}$)	Seeds/m^2
Poa pratensis	Rhizomes	6.8	32,225
Festuca brevipila	Bunch type	22.7	28,275
Festuca arundinaceae	Bunch type/short rhizomes	27.2	14,513
Lolium perenne	Bunch type	27.2	14,811

random effect to account for repeated measures throughout the sampling season, and a Tukey means separation protocol was used if F statistics were less than or equal to $\alpha = 0.05$ to test for significant differences among factor levels. The bloom response variable was analyzed with a linear regression model without mixed effects as a result of the blooms occurring only during one time point in the year (July). Assumptions of homoscedasticity and normality were verified by inspection of residual plots. Square root transformations were sufficient to meet assumptions for all analyses except for the bloom response variable in trial 1, in which case a log transformation was used.

Results

Trifoliate leaf count and percent cover of Kura clover were highly correlated (r = 0.937, $t_{(214)} = 39.2607$, $P < 0.001$), so only trifoliate leaf counts were used in the analysis as a result of the qualitative nature of the leaf cover measurement. The relationship between trifoliate leaves and bloom number was strong (r = 0.712, $t_{(70)} = 8.4764$, $P <$ 0.001), indicating trifoliate leaf count is a good predictor of bloom count.

As result of the significant effect of trial in our initial model, trials were analyzed separately. In both trials, turf species affected significantly the numbers of trifoliate leaves and blooms ($P \leq 0.05$) (Table 2.). The seeding rate of Kura clover did not affect leaf or bloom counts significantly in both trials, and no significant interaction between turf species and seeding rate was detected. Hence, all pairwise comparisons between trifoliate leaves and blooms in different grass treatments were averaged over Kura clover seeding rate.

Trial 1. The average number of Kura clover trifoliate leaves in kentucky bluegrass ($\mu = 352$) and hard fescue ($\mu = 365$) plots were significantly greater than in both tall fescue (μ = 132) and perennial ryegrass (μ = 151) plots (Fig. 1A). This pattern was similar for the average number of blooms in the plots, with kentucky bluegrass (μ = 24) and hard fescue (μ = 26) both having significantly greater average Kura clover bloom counts than tall fescue (μ = 3) and perennial ryegrass (μ = 2).

Trial 2. The average number of trifoliate leaves and blooms in kentucky bluegrass plots (μ = 121 and 17, respectively) were significantly greater than average trifoliate leaf and bloom count in both tall fescue (μ= 57 and 3, respectively) and perennial ryegrass plots (μ = 63 and 4, respectively) (Fig. 1B). Unlike trial 1, hard fescue had intermediate numbers of average trifoliate leave and bloom counts (μ = 91 and 11, respectively), and was not significantly different from perennial ryegrass, tall fescue, or kentucky bluegrass.

Discussion

Kura clover establishment and bloom were affected by turfgrass companion species but were unaffected by seeding rate. Establishment and bloom were greatest in kentucky bluegrass when compared with tall fescue and perennial ryegrass, despite the differences in establishment between years. Kura clover tended to have greater establishment and bloom abundance in hard fescue than in perennial ryegrass or tall fescue, but was only statistically greater in the first year.

The well-known slow establishment rate of kentucky bluegrass (Christians et al., 2017) may have contributed to this result; the cultivar we used in this study, 'Moonlight SLT', exhibits poor seedling vigor compared with other kentucky bluegrass cultivars (Morris, 2016). Another factor that may have contributed to the greater establishment of Kura clover in kentucky bluegrass was the limited fertilizer regime used in our study. A previous study from Wisconsin found kentucky bluegrass stands to be especially weed prone compared with other turf species, but showed decreasing weed cover with increasing nitrogen rates (DeBels et al., 2012). The low rate of fertilization in our study may have resulted in a slower growth rate of kentucky bluegrass, and gave Kura clover more opportunity to establish. The fertilizer requirements for grass establishment in our plots were likely on the low side of recommended rates as a result of the loamy soil, conservative cutting regime, and the fact that clippings were left on the field.

There was lower establishment of Kura clover in trial 2 compared with trial 1 despite efforts to keep establishment conditions (i.e., nutrient availability and soil moisture content) consistent. Kura clover is known to be highly variable in its establishment (Seguin et al., 1999) and was noted as being potentially sensitive to establishment conditions. Climatic conditions and time of establishment between trial years were somewhat different, with average temperatures during the first week of establishment being somewhat higher during trial 1 than trial 1 (2 °C for high temperatures and 4 °C for low temperatures). This higher average temperature during trial 1 may have been responsible in part for the greater rates of establishment compared with trial 2. Soil testing for both

Table 2. Analysis of variance results conducted for trial 1 established in 2013 and trial 2 established in 2014. Within each trial, we tested for the effect of grass species (GS), seeding rate (SR), and their interaction on average Kura clover trifoliate leaf and bloom count.

Factor	df	*F* value	*P* value
Trial 1			
Trifoliate leaves			
GS	3	21.73	<0.001*
Kura SR	2	2.05	0.151
GS × SR	6	0.83	0.557
Blooms			
GS	3	2.97	<0.001*
SR	2	0.19	0.133
GS × SR	6	0.14	0.302
Trial 2			
Trifoliate leaves			
GS	3	4.03	0.019*
SR	2	2.68	0.089
GS × SR	6	0.87	0.529
Blooms			
GS	3	6.95	0.002*
SR	2	0.29	0.748
GS × SR	6	0.89	0.515

*Significant result.

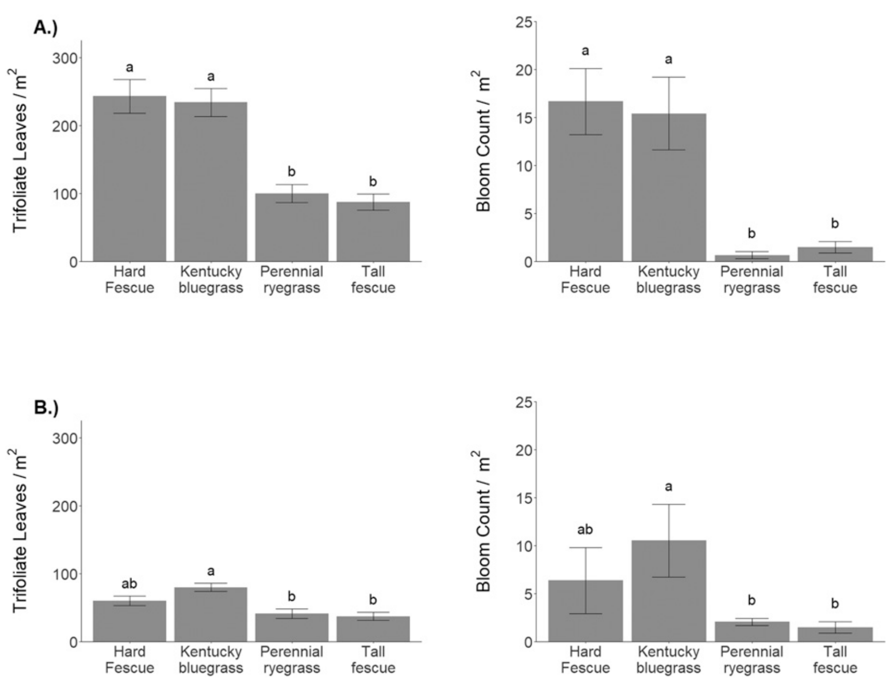

Fig. 1. (**A**) Bar plots of mean seasonal Kura clover blooms and trifoliate leaves for trial 1 in 2014 and (**B**) trial 2 in 2015 in four different grass species. Error bars represent SE and letters represent statistical differences as determined by pairwise comparison using Tukey's mean separation protocol with α = 0.05.

trials revealed that the nutrient conditions and organic matter for the two trials were strikingly similar, and thus fertility as a primary driver for differences between trials seems unlikely (see Supplemental Table 1 for soil analysis).

One potential explanation for lower Kura establishment could be the result of greater levels of competition. The field space where trial 2 was established was prepared for planting in 2013 through tilling and leveling, but was left fallow for 1 year without further cultivation. Although direct measurements of weed pressure were not taken as a part of this study, it is possible this fallow period without further cultivation might have led to a larger weed seed bank, and thus greater weed pressure on plot establishment in trial 2 (Froud-Williams et al., 1983; Roberts and Dawkins, 1967). This weed pressure may have also interfered with the establishment of the turfgrass species as well, but we were unable to assess this because we did not collect data on turf establishment past visual confirmation.

Although weed pressure may have been different between trials, the relatively small footprint of the research area and the randomization process of the plots was likely consistent enough across a given trial not to interfere with seeding rate and grass species treatment analysis within a given trial. Thus, our consideration of each trial separately addresses issues of potential differences in weed pressure between trials adequately. It is possible that weed pressure may have affected Kura clover establishment indirectly through competition with the different turfgrass companion species, but this is impossible to tell from our data. Although Kura establishment was less on the whole in trial 2, the pattern of establishment was somewhat consistent between trials, with the most glaring difference being that Kura clover establishment in hard fescue was not statistically different from the other grass species in trial 2. Although Kura clover establishment does trend higher than in perennial ryegrass and tall fescue, it is likely that a combination of environmental factors interacting with hard fescue in trial 2 modified its competitive interactions with Kura clover. Fine fescues are known to suppress weed growth, (Bertin et al., 2003, 2009), and there is some evidence this suppression relies on environmental conditions (Bertin et al., 2009).

Other studies have found effects of turfgrass species on forb establishment. A recent study involving white clover co-seeded with companion grasses into dormant bermudagrass found clover produced more trifoliate leaves in tall fescue compared with the faster growing varieties such as annual ryegrass (McCurdy et al., 2013). A similar study used three species of turfgrass co-seeded with birdsfoot trefoil (*Lotus corniculatus* L.), and found that kentucky bluegrass and red fescue typically had greater yields of birdsfoot trefoil than perennial ryegrass (Laskey and Wakefield, 1978). These studies generally support the hypothesis that slow-growing grasses are more amenable to the growth of companion forbs, although there is also evidence that tall fescue is partially allelopathic to species in the clover genus (*Trifolium*) (Springer, 1996). Our results generally support these findings, but have expanded them to include flowering response, suggesting that grass species could be an important consideration for managing turf for high floral densities. Although our study was constrained to Upper Midwest of the United States, the number of studies with similar results span many climatic zones, suggesting this relationship could be applicable in a range of systems.

Mowing and public use have been found to affect lawn community diversity negatively (Bertoncini et al., 2012; Shwartz et al., 2013), but the species of turfgrass used in lawns also has an important impact on whether flowers can establish and continue to bloom in lawns. Our study combined with other previous research suggests that slow-growing turfgrasses such as kentucky bluegrass and hard fescue could be good candidates for future lawn forb trials aimed at identifying new species for flowering lawn mixes, especially those that are slow growing and have challenging germination requirements. One important factor we did not explore is how populations of flowers might change as turf swards mature, whether their populations are truly sustainable in the long term, and whether grass species impact this. Long-term stability of flower populations in turf lawns is key to the goal of maintaining diversity and an important avenue of future research. Ultimately, finding commercially available flowers that can tolerate mowing, create acceptable aesthetics, provide bee forage, persist long term, and meet the needs of landowners is a challenge. This work attempts to address one of these challenges; our future studies will build off these results to provide useful forb–lawn mixtures.

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Supplemental Table 1. Soil test.

Laboratory ref. no:	Soil job #125 2015–16					Research Analytical Laboratory
Report to:	Ian Lane					University of Minnesota
Date received:	3/10/2016					1902 Dudley Ave.
Date reported:	3/17/2016					St Paul, MN 55108
Study name:	Bee Lawn					
Sample type:	Soil					
Laboratory no.	Sample ID	Bray P (ppm)	NH_4OAc-K (ppm)	LOI OM (%)	Water pH	
1	Trial One	101 / 101	274 / 278	4.6 / 4.6	6.5 / 6.5	
2	Trial Two	105	286	4.5	6.1	