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DELVING DEEPER INTO PLANT ASSEMBLY: DETERMINING THE ROLE OF INTRASPECIFIC DIVERSITY ACROSS SPACE AND TIME TO AFFECT CARBON DYNAMICS IN NATURAL AND MANAGED SYSTEMS

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DELVING DEEPER INTO PLANT ASSEMBLY: DETERMINING THE ROLE OF INTRASPECIFIC DIVERSITY ACROSS SPACE AND TIME TO AFFECT CARBON DYNAMICS IN NATURAL AND MANAGED SYSTEMS

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BY

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Abstract

Understanding the key ecological processes contributing towards the spatial-temporal variation in intra-specific diversity of commonly occurring species, especially during early colonization events, can be key to predict how temperate prairies will be assembled and will function in the future. Specifically, little is known about the role of plant-plant interactions during population assembly following disturbance events to shape intra-specific variation/diversity and overall plant productivity. Priority effects state that individuals that arrive first will have advantages over late arriving, such as first access to nutrient and space for growth, which affect the later assembly of subsequent other individuals. This experiment will test whether priority effects play a role determining genotypic and phenotypic intra-specific variation and diversity in five plant species commonly occurring in the Southern Great Plains. To test the effects of priority effects modulating intra-specific variation and diversity across space and time, I collected plant species across nine sites along climatic gradient. Then I tested how phenotypic distance influence plant-plant interactions during early colonization to determine intra-specific variation and diversity. The results will help determine the role of plant-plant interactions during colonization to influence population assembly as it does community assembly playing a key role determining intra-specific diversity. These research results will strengthen our understanding of how diversity within species comes about and influences the rest of the community to shape ecosystem biodiversity and function, and may help us better manage the prairie habitat. Assembly is an important component of agriculture. In modern agriculture, crops are replanted every year. To do this a field is cleared or disturbed to become ready for the crop. The crop then assembles. The disturbance and crop assembly shape the new ecosystem and change it in drastic ways especially with nutrients in the soil. This study aims to understand how different treatments (annual, perennial, and restored prairie) will affect the soil carbon, nitrogen, and organic matter. Soil was collected at 0-15cm and 15-30cm for each treatment. Soil was then incubated for 120 days to determine the CO_2 evolution and cumulative CO_2. Soil was tested externally for nitrogen and soil organic matter. These results can help us better understand the effect of assembly on soil and offer a way to better manage agricultural soils through crop type.

Chapter 1: Determining the role of early vs. late colonization of plant genotypes to affect intraspecific variation and diversity across space and time

Introduction

Community assembly, or the order in which species arrive after a disturbance, can strongly influence the variation in species' distribution, diversity, and composition across space and time (Chase, 2003). Generally, individuals that arrive first have an advantage over later arriving individuals, greatly benefiting from early colonization (Fukami, 2015; Fukami *et al*., 2016). These advantages are referred to as priority effects. The strength of the priority effect can be influenced by the environment and fitness of a species (Fukami et al., 2016, Leopold et al., 2017, Tucker and Fukami, 2014). Fukami *et al*. (2010) found that when looking at wood decomposing fungi, early arrival played a larger role in influencing the final community composition than environmental properties (e.g., nitrogen availability). Another study looking at wood decomposing fungi showed that the strength of the priority effect was influenced by resource availability (nitrogen, a limiting resource) and the amount of fungivore grazers (Leopold *et al*., 2017). Similarly, grassland plant species experienced greater priority effects at high nutrient supply than low nutrient supply (Kardol *et al*., 2012). Besides resources, whether an individual is exotic or native (species origin), can also play a significant role in how species benefit and/or tolerate priority affects (Wisley *et al.*, 2015).

Native plants provide a variety of ecosystem services by preforming nutrient cycling, water filtering, as well as animals' habitats and food (Eviner and Chapin, 2001). Native plants spend many millennia evolving to fit within an environment (Simberloff *et al*., 2012). They have also co-evolved with other above- and belowground biota, which work together to perform certain ecosystem functions. Exotic species will often come into an ecosystem and decrease the number of ecosystem functions performed (Vitousek *et al*., 1997, Ricciardi *et al*., 2013). Exotics can also alter the environment, by changing soil legacies (properties through time), which makes it harder for natives to establish (Grman and Suding, 2010). Some exotics appear to have the ability to suppress native plants, but if the natives have the advantage of priority effects, they may be able

to mitigate some of these suppressing effects (Suding *et al*., 2013, Vaughn and Young, 2015). Grman and Suding (2010) found that priority roles largely affected whether the exotic or native would be more successful and generate more biomass. Early germination may have lasting effects, allowing natives to withstand exotic invasions (Vaugn and Young, 2015). Unfortunately, priority effects do not mitigate all exotic effects. Often exotics are less negatively impacted by priority effects since they have higher germination and earlier emergence than natives (Stuble and Souza, 2016, Wilsey *et al*., 2015). More research has been done to understand how early arrival is affected by interspecific diversity including comparing invasive and natives and how they shift ecosystem function, but the influence of intraspecific diversity populations during early arrival is less well understood. Since native and exotic species have different life histories and ecosystem function, it seems like they would respond differently even when grown intraspecifically.

Intraspecific diversity shapes ecosystem structure and functions and is an important facet to increasing our understanding of biodiversity (Hughes *et al*., 2008). While intraspecific diversity is often studied from an evolutionary point of view, this does fully explain how inherited genes and traits within a population can influence other individuals, species, and the ecosystem as a whole (Bolnick *et al*., 2011). Crutsinger *et a*l. (2006) were the first to demonstrate how increasing the number of plant genotypes not only increased primary production, but also positively influenced species richness of higher trophic levels (arthropod herbivores and predators). Johnson (2008) further supported this when he found that plant genotypes influenced other tropic levels. Plant genotypes with higher water use efficiency and lower leaf nitrogen had higher aphid densities. Higher aphid densities then supported higher aphid-tending ant abundances.

Much of the intraspecific research is aimed at understanding how intraspecific variation can function like interspecific diversity in ecosystem, but not how invasive and natives respond to intraspecific diversity in relation to priority effects. Genotypic diversity may influence ecosystems differently over time. Souza *et al*. (2017) found that *Solidago altissima* genotypes only influenced carbon dynamics in the early growing season, while water dynamics were

significantly influenced later in the growing season. Intraspecific diversity may not always be the best predictor of what will influence ecosystem function and structure. Sometimes certain genotypes are better suited or more fit for an environment and perform better than a diverse group of less suitable genotypes (Fischer *et al*., 2017; Smith *et al*., 2011). For example, many exotic plants are generally more suited to invade areas. When comparing genetic differences of natives and exotics by using genotype sizes, natives tended to have larger genomes in their shrubs and lianas than invasives (Fridley and Craddock, 2015). The smaller genomes could help invasives invade since plants with smaller genomes typically have greater growth rates than species with larger genomes (Pandit *et al*., 2014).

Our study assesses the impact of priority effects on influencing intraspecific variation and diversity with associated ecosystem functions in native vs. invasive grass populations. To do this we collected four species of grasses (natives: *Sorghastrum nutans* and *Schizachyrium scoparium*; exotics: *Sorghum halepense* and *Bothriochloa ischaemum*) across nine sites throughout precipitation gradient of Oklahoma. Each collected plant was treated as if it were a unique genotype. We then established mesocosms with one or two genotypes, as well as controls with three genotypes. After 21 days, we added plant genotypes to the mesocosms with one or two genotypes, so that each mesocosm had a total of three unique genotypes. To understand the magnitude of priority effects, we quantified individual-level and mesocosm-level biomass production, and to quantify the magnitude of priority effects on ecosystem function we measured net ecosystem CO_2 exchange (NEE). We asked (1) Do native or exotic population receive greater benefit from early arrival or less negative effects from late arrivals? (2) Does increasing phenotypic distance also increase plant genotype performance (biomass) in exotic more than native populations? (4) Do natives or invasives create more biomass and is the biomass correlated with NEE? We predicted that exotics would benefit more from early arrival and be less negatively impacted by late arrival. We also predicted that increasing phenotypic distance would increase biomass, and the exotics would create more biomass, but have less NEE.

Methods

Plant Collections

We collected four graminoid species across nine sites that varied in annual precipitation and temperature (Table 1). Collections occurred in late summer 2017. The four grass species consisted of two native grasses (*Sorghastrum nutans* and *Schizachyrium scoparium*) and two non-native invasive species (*Sorghum halepense* and *Bothriochloa ischaemum*). We collected six individuals of each grass species at each of the nine sites and recorded plant trait data and GPS location for each individual collected. We measured plant height (cm), and collected a mature leaf and inflorescence to quantify foliar traits and inflorescence mass. Each foliar sample was scanned to obtain leaf area (cm^2) using winFOLIA software (Regent, Quebec, Canada). The leaf was then oven dried the leaf at 65°C for approximately 48 hours before having its dry mass weighed. The specific leaf area was determined by the dividing the mass by the area. To quantify the leaf dry matter content (LDMC), the fresh mass of a leaf was measured, then the leaf was oven dried at 65°C for approximately 48 hours. The LDMC is the dry mass divided by the fresh mass. To quantify belowground traits, we subsampled ten fine root hairs that were scanned, and the specific root length determined WinRhizo (Regent, Quebec, Canada).

Experimental Design

To start, we created a Bray-Curtis similarity matrix for each species. The Bray-Curtis similarity matrix used the plant field trait composition to find the phenotypic similarity between each individual. For each species, a Principle Components Analysis (PCA) was performed using the Bray-Curtis matrix to visualize the phenotype similarity between individuals (Appendix A). The PCA was used to determine how similar the individuals were based on how close they are on the axes determined by the two principle components. For each species, four plants were chosen based on their PCA relatedness and if they had many tillers (more than 30 tillers). Plants were chosen with various phenotypic similarities ranging from low to high different in ordination space.

The parent plant with the corresponding phenotype was selected and individual tillers, or clones, from the parent plant were propagated to replicate that phenotype/genotype as we prepared our

mesocosms. For each of the four plant species, our mesocosm treatments included: (1) Control (3 individuals, n=4), (2) Early & Late arrivals (1 early arrival with 2 late arrivals n=12 , 2 early arrivals with one late arrival n=12 pots), N=28. Controls were created that started with three individuals each with different genotype. After twenty-one days, tillers were added as "Late Arrivals" into mesocosms containing each a single or two other tillers. For instance, If a mesocosm had two tillers, then one tiller with a different genotype was added. If a mesocosm had one tiller, then two tillers both with unique genotypes were added.

Grasses were allowed to grow together for the next five months. During this time, the following trait data were gathered. To better understand ecosystem function, we measure the carbon flux through net ecosystem exchange (NEE, μmols CO_2 m^{-2} s^{-1}). To do this measurement, a single pot was placed under a portable chamber made of semi-transparent plastic and PVC pipe. A LI-COR 7500 infrared gas analyzer (Licor Inc., Lincoln, NE, USA) was attached to the chamber and the flux between carbon dioxide (CO_2) and water vapor (H_2O) for 120s (Arnone and Obrist 2003) recorded. This was repeated for all experimental pots. At the end of five months, above- and belowground biomasses were determined. For the aboveground biomass, each individual was clipped and placed in a paper bag. The individual was dried at 65°C for 48 hours before the dry mass was weighed. The below ground biomass was calculated by removing all of the roots from the pot and removing as much soil as possible before rinsing them. The rinsed roots were then placed in a paper bag and dried at 65°C for 48 hours before weighing.

Statistical Analyses

To determine the magnitude of the priority effects, we calculated the standard effect size (Gurevitch and Hedges, 1999) by the log ratio of the total biomass (above- and belowground) under early/late arrivals (as numerators) and controls (as the denominator). The mean calculated effect size had 95% confidence intervals that were calculated around them. This allowed us to compare the effect of early and late arrival on the different species. Regression was used to determine the relationship between phenotypic neighbor distance and biomass. A regression was used to determine the NEE with total biomass. To see if there was any significant difference of total biomass or NEE among the species, we did an ANOVA.

Results

Priority Effects: Early vs. Late Plant Genotype Arrival

Plant genotypes, across populations of native and exotic species, were similarly negatively impacted by late mesocosm colonization, whereas the impacts of early colonization on plant genotype performance (total biomass) depended on specific populations. *Schizachyrium scoparium* was negatively impacted while *S. halepense* was slightly positively impacted by early arrival relative to the control (Fig 1). On the other hand, the effect of early colonization of *S. nutans* and *B. ischaemum* plant genotypes was not significant. As we compared whether plant populations differed by early arrival, *S. halepense* and *S. scoparium* had populations (mesocosms) that significantly differed from each other ($F=2.54$, $p = 0.06$). Finally, plant genotypes across all populations were negatively impacted by late arrival (Fig 2). We found no significant difference between the magnitude of the negative impact on late arrival across all four populations; with all populations of each species responding similarly to delayed colonization (ANOVA, $F=0.03$, $p = 0.99$).

Phenotypic Neighbor Distance: Influence on Plant Genotype Performance

Phenotypic distance did not affect biomass in any of the species' regressions when graphed except for *S. scoparium* (Fig 3). When running a regression, a significant effect was picked up for *S. scoparium*, where more distance phenotypes had less biomass ($R^2 =0.11$, $p < 0.01$). The ANOVA did not show any relation between biomass and phenotypic distances for the other species (all $p > 0.05$).

Mesocosm Function: Biomass and Carbon Exchange

Mesocosm productivity (above and belowground biomass) and carbon dioxide exchange differed significantly across plant populations. Generally, mesocosm populations composed of exotic genotypes produced more biomass (ANOVA, $F = 111.60$, $p = <0.01$) and exchanged greater carbon dioxide (ANOVA, $F = 7.71$, $p < 0.01$) than native genotypes. For example, exotic populations produced 385% greater total biomass than native populations. Similarly, exotic populations exchanged 42% greater total CO_2 than native populations (Table 2).

Linking Changes in Mesocosm Biomass to Changes in Carbon Exchange

Generally, increases in mesocosm biomass were not necessarily related to changes in net ecosystem CO_2 exchange. In other words, as mesocosm biomass increased, carbon exchange did not necessarily increase across plant populations. The NEE and biomass were marginally significantly correlated only for *S. halepense*, where the NEE increase with increasing biomass (R^2 =0.14, p = 0.05)(Fig 4). None of the other species had a relationship between their biomass and NEE (R^2 <0.05, p > 0.05). Overall, *S. scoparium* had much less NEE than the other species (Table 2).

Discussion

Priority effects were explored by looking at their influence on the performance of total biomass and NEE on four prairie grasses. The four species were differed by species origin (native: *Sorghastrum nutans* and *Schizachyrium scoparium* vs exotic: *Sorghum halepense* and *Bothriochloa ischaemum*). We were also interested in how intraspecific diversity would influence their performance so mesocosms were set up with only an individual species. To simulated priority effects new individuals were added to the mesocosms 21 days later. The benefit or cost of priority effects did not seem to be influenced by species origin: native or exotic. We documented mixed results in the early arrival benefits with positive, negative, and neutral effects exhibited by plant genotypes in native and exotic populations alike; while plant genotypes across all populations, native and exotic, were negatively affected by late arrival. Further, phenotypic distance from neighbors did not appear to influence plant genotypic performance except in *S. scoparium* where biomass decreased with increased phenotypic neighbor distance. The mesocosms consisting of exotics did have greater biomass and net ecosystem CO_2 exchange (NEE) than the native mesocosms. Surprisingly, mesocosm total biomass and NEE did not correlate with each other except weakly for *S. halepense* genotypes.

The response of the native and exotic populations to early arrival was different from what we expected based on the literature . Xu et al(2015) found when exotics are grown together in common garden, they have earlier emergence than natives grown together. Due to this early emergence, exotics also grew more quickly and created more biomass during the early growing

season. This higher biomass allowed the exotic populations to be more resistant to plant invasions than native populations, but there was not a statistical difference in plant invasions between exotic and native populations over the full growing season. Natives have been found to do better in the later season since they have greater niche complementarity that allows them to utilize more resources than exotics (Isabel and Wilsey, 2011). The Stuble and Souza (2016) experiment, addressing priority effects for native and exotic plant species, found that grassland exotics had an advantage over natives when arriving late but did not differ with early arrival. In other words, exotic species paid a lower cost than natives with delayed arrival in a grassland community. Wilsey et al. (2015) found when comparing regeneration ability for exotic and native grasses that exotics had higher germination of seeds than natives, likely exhibiting greater advantage than native counterparts. Higher germination may be why exotics typically experience less negative effects when arriving late. Wilsey et al.'s (2015) exotic communities also had more biomass than the native communities, which prevented late arriving species from doing as well in the exotic mesocosms when compared to the native mesocosms. Schizachyrium scoparium was the only species that was negatively affected by early arrival compared to the control. The S. scoparium controls may have performed better due to facilitation since native plants are known to facilitate each other, leading to positive complementary effects (Kuebbing et al., 2015). Finally, previously mentioned studies had mesocosm assemblies with both natives and exotics competing in the same pot, while our study had each species competing with itself (e.g., intraspecifically). When invasives and natives are grown together, the invasives seem to be able to suppress natives, but natives may not possess the same ability to suppress invasives (Maron and Marler, 2008).

The late arrival being similarly negative may have been due to each species having little intraspecific variation. Crutsinger *et al*. (2009) found that different species of *Solidago* (interspecific variation) had much stronger effects on leaf litter decomposition and nitrogen cycling than genotype diversity (intraspecific diversity). When there is interspecific diversity, there is a greater difference in genotypes species, which allows the different individuals to interact differently with the environment, which also influences the species interactions. Another reason exotics are thought to have an advantage with early arrival is that they often germinate

earlier in the growing season than natives (Wainright *et al*., 2012). Since all of our individuals were taken from parent plants, the exotics did not have a sprouting early advantage to the natives.

Phenotypic neighbor distance did not affect biomass in most species, with only a weak negative correlation between phenotypic distance and biomass for *S. scoparium*. Crutsinger *et al*. (2016) found that when assemblages had greater intraspecific diversity, the overall biomass of the mesocosm increased. Other studies had either mixed or no significance when looking at biomass in relation to intraspecific diversity (Souza *et al*., 2017, Diaz *et al*., 2007, Fisher *et al*., 2017, Cadotte *et al*. 2013). Norberg *et al*. (2001) did not uncover strong correlations between intraspecific diversity and biomass alone, but found a much stronger relationship between phenotype, biomass, and environmental factors, particularly how the environment changes over longer periods of time. While Crawford and Whitney (2010) had weak correlation between biomass and plant genotype, they found that when genetic diversity was analyzed together with a combination of other traits (seedling emergence, flower duration, and reproduction), it had a greater positive affect than when each variable was correlated alone. Souza *et al*. (2017) found that the presence of certain genotypes was a better predictor of biomass than the comparison of single genotype monocultures to multiple genotype polycultures. Any of the previous factors may explain why we did not discover any strong relationship. Our experiment was over a relatively short duration of time (about 5 months), so there may not have been enough time. Potentially certain genotypes were able to produce more biomass than others or perhaps the duration of the experiment was not long enough to see a divergence in the biomass Potentially there would have been a stronger relationship with genetic distance and biomass if other variables were considered too.

The total biomass of the exotic species (*B. ischaemum* and *S. halepense*) was greater than the natives (*S. nutans* and *S. scoparium*). Exotic grassland species have been found to have greater biomass (aboveground and seedling) than native species (Ehrenfled 2003, Smith *et al*. 2000, Wilsey *et al*., 2015). Smith *et al*. (2000) found that native annuals have less aboveground biomass than invasive *Bromus*, and the gap increases when carbon dioxide is elevated. Kuebbing

et al. (2015) determined that invasives had more aboveground biomass, but in contrast, natives had more below ground biomass. Our results diverge slightly since the exotics had both more above- and belowground biomass. Biomass was correlated with NEE only for *S. halepense*. While Zou *et al*. (2007) did not correlate biomass and net carbon dioxide assimilation, both variables were significantly higher in invasive *Sapium sebiferum* populations than native populations. Mielnick *et al*. (2005) discovered that grassland species had the largest biomass in the month with the greatest carbon dioxide uptake, but the biomass was not correlated with annual carbon dioxide fluxes over 4 years. Soil water may be a better predictor for biomass for some grassland species than gas exchange. Potentially our species did not correlate biomass and NEE because their biomass may be more linked to water amount.

Conclusions

When grown with other individuals of the same species, natives and invasives are similarly negatively impacted by late arrival, contrary to our original prediction. We also predicted that exotic plant genotypes would exhibit greater priority effects than native ones and benefit from early arrival; however, we found mixed results with benefits costs for early arrival by native and exotic genotypes. Also, surprising and different from our predictions, the phenotypic distance to neighbors did not influence plant genotype performance, perhaps due to a short phenotypic distance to neighbors. The exotic assembled mesocosms generated more biomass than the natives and often showed greater carbon dioxide exchange. However, the biomass of a given mesocosm was generally not correlated with its carbon dioxide exchange. Taken together, priority effects during population assembly may play a smaller role in determining the performance of divergence genotypes within a species than it does when comparing across species during community assembly.

Chapter 2: The effect of plant cultivation type and soil depth on soil carbon and nitrogen dynamics

Introduction

As the global demand for food increases, humans continue to clear natural landscapes and work to increase production of food (Tilman *et al*., 2011). Often these natural landscapes are converted from complex networks of predominantly perennial plants to a single annual crop called a monoculture (Matson *et al*., 1997). As these complex networks become simpler ecological communities, soil microorganism systems are altered, which can lead to changes in carbon and nitrogen cycling (McLauchlan *et al.,* 2006). Humans also disrupt the soil through tilling, which exposes carbon and nitrogen that would normally be securely stored (Bronick and Lal, 2005). This disruption allows annual crops easy access to soil carbon and nitrogen, but changes in soil structure lead to a loss of carbon and nitrogen through erosion and increased microbial decomposition. When the carbon and nitrogen are gone, plants are unable to grow in that area since they need carbon and nitrogen to create new cells. In contrast, most natural systems, especially perennial dominated ecosystems, tend to store carbon and nitrogen over time. One potential management solution for protecting soil carbon and nitrogen while continuing to create food is to grow perennial crops instead of annual crops.

One critical soil property altered through greater cultivation intensification is soil organic matter (SOM). SOM mainly consists of organic animal and plant material and humus (Lehmann and Kleber, 2015). The SOM is needed for physical, chemical, and biological functions within the soil, which increase plant productivity (Dalal *et al*., 2011). The organic materials contained in SOM include carbon, nitrogen, and other nutrients (Schmidt *et al*., 2011). Soil organic carbon (SOC) is the largest component of SOM, comprising approximately 50% of its total mass. SOC consists of labile and recalcitrant carbon pools. Labile carbon is composed of carbohydrates and proteins. It is often in surface soils and cycles quickly (Rovira and Vallejo, 2002). The rate of cycling depends partially on exposure to air and is influenced by the amount of organic material added each year. Recalcitrant carbon is composed of mostly of lignin, which cycles slowly and is resistant to decomposition (Hoyle *et al*., 2011). If the amount of carbon exported is greater than

the amount of carbon returned, SOC, especially the labile carbon, will decline. This decline often occurs in cropping systems and can lead to a loss in soil resilience or ability to recover after a deterioration event.

Soil depth can greatly influence the amount of carbon in the soil. While there is some debate on whether surface soils or deeper soils (1m+) have greater carbon pools, it is recognized that new organic matter enters the soil through surface soils (Harrison *et al*., 2011; Cambardella, 2005). Most soil carbon initially starts as decaying plant and animal matter, which once decomposed is referred to as organic matter. This organic matter is mostly integrated into the surface soils, but some organic matter leaches or is mixed by animals into deeper layers (Cambardella, 2005). Plants' roots can also sequester carbon deeper in the soil (Monti and Zatta 2009). For example, perennials have roots that grow much longer and have greater biomass in deeper soils than annuals, which allows them to sequester more carbon deeper in the soil profile. Vegetation type often influences SOC distribution through the soil profile (Jobbagy and Jackson, 2000). For grasslands, about 42% of the soil organic carbon is held in the first 20cm while forests have about 50%.

Beyond vegetation composition, cultivation type also leads to difference in the amount of SOC. Some cultivation types include tilling, which is a disturbance that can cause much of the soil's stored carbon to be exposed to oxygen and quickly broken down by microbes, decreasing the soil's carbon stores. When a cropping system was converted to un-tilled pasture, the soil increased in carbon a greater amount in shallow soils (40%) than in deeper soils (10%) (Guo and Gifford, 2002). Perennial crops had more carbon in the A horizon (0-25cm) than conventional annual cropping systems that included tilling (Syswerda *et al.*, 2011).

Our study evaluates the effect of agricultural intensification at three different cultivation types: annual crop, perennial crop, and restored prairie at both upper (0-15cm) and lower (15-30cm) soil depths. We established a laboratory soil incubation experiment to quantify how soil CO_2 evolution (as a proxy for microbial activity and C mineralization). If there is greater CO_2 evolution, then the soil has more carbon stored that can be utilized by microbes. Samples were

analyzed externally for SOM, soil nitrogen, and soil C:N are impacted by annual vs. perennial crop assembly at shallow and deeper soil depths. Specifically, we asked the following questions: (1) Do cultivation type and soil depth affect soil CO_2 evolution? (2) How do cultivation type and soil depth affect soil properties such as total C and N, C:N, and SOM? We predicted that less managed cultivation like the restored prairie would exhibit the greatest CO_2 evolution due to having more carbon, nitrogen, and SOM than a highly managed cultivation type like the annual crop. We also predicted that upper layers of soil would have greater CO_2 evolution due to greater total carbon, nitrogen and SOM than lower layers of soil.

Methods

Study Site and Experimental Design

Soil collections took place at the Land Institute in Salina, Saline County, Kansas, USA (38.7684° N, 97.5664° W) in June 2018. The mean annual temperature is 12°C, and mean precipitation is 819mm. The soil is classified as coarse-silty mixed, mesic Fluventic Haplustoll (Oliveira *et al*., 2018). The Land Institute has an experimental field with 900m^2 plots at three different levels of agricultural intensification established in 2002 from previous alfalfa field. No intensification does not have any human inputs, minimal intensification is tilled every 3-5 years, and heavy intensification is tilled every year. A randomized block design was set up that included three plant cultivation treatments: (1) restored prairie (no intensification), (2) perennial intermediate wheatgrass, *Thinopyrum intermedium*, (minimal intensification and precursor to the perennial grain, Kernza), and (3) annual wheat, *Triticum aestivum,* (heavy intensification). Each plant cultivation treatment type has three replicates for nine total plots across all treatment combinations. The cultivation treatments aimed at addressing how a perennial-dominated system performs relative to annual in terms of plant characteristics (root:shoot ratio) and ecosystem properties (C and N cycling) (Crews et al. 2016). Plant traits such as biomass and productivity rely on nutrients within the soil (Chiariello *et al*., 1980). Carbon is a building block for plant tissue and nitrogen is often a limiting nutrient for plant growth (Chiariello *et al*., 1980, Agren *et al*., 2012).

Soil Sampling and Incubation

In each experimental plot, we sampled at three randomly selected locations, collecting soil cores (2.5 cm diameter × 15 cm in length) at two depths: (1) 0-15cm and (2) 15-30cm. The soil was homogenized in a ziplock bag. Then the soil was sieved through a 2mm sieve. Then a subsample 30 g of soil was placed in 50mL falcon tubes and mixed with 3.6 mL water. (to bring it to approximately 60% of its water holding capacity) and kept at ambient temperature of 25°C (DeGraaff, 2010). A separate falcon tube was filled with 10 mL of distilled water to help maintain humidity throughout the incubation. Then, a microcosm was created by placing a soil tube and a water tube in a mason jar and then closing the system with a screw on lid. The jars were placed in a dark place and incubated for 120 days.

We recorded the CO_2 evolution on the initial day (day 0) as well as day 1, 2, 3, 5, 8, 15, 30, 60, 90, and 120 using a Li-COR 6400 infra-red gas analyzer (LiCOR, Lincoln, Nebraska) for each jar. We used a syringe to extract 15 mL of gas, which was injected into the Li-COR 6400 tubing for 45 seconds per a sample. Each jar was allowed to breathe with the lid off for 30 minutes after its CO_2 measurement was taken.

To determine the CO_2 evolution, first the area under the curve was calculated with KaleidaGraph by plotting CO_2 recorded values for 45 seconds. Using this data, graphs were created to show the change of CO_2 over time in days. The area under the curve was converted to adjusted ppm by multiplying the slope from the standard curve of a blank and adding the standard curve intercept. The ppm was then converted into μg CO_2-C L^{-1} by multiplying parts per million (ppm) by the atmosphere and molecular weight CO_2-C and dividing that number by the gas constant multiplied by the temperature in Kelvin. Finally, μg CO_2-C g^{-1} was found by multiplying ug CO_2-C L^{-1} by the jar's volume and dividing it by the dry mass of the soil.

Soil Organic Matter and Nitrogen

To determine soil organic matter and nitrogen, we allowed soils to dry for 48 hours at 105° C. Then the soil was put through a 2 mm mesh sieve to remove rocks, roots, and macro-fauna before being ground with a mortar and pestle. The ground soil was sieved again through a

0.25mm mesh sieve. Ten grams of finely ground soil was collected and placed in a ceramic bowl. The soil carbonates were dissolved by adding drops of 1N HCL until the soil was saturated. The soil was left at room temperature for 24 hours to remove the carbonates.

After the carbonates were removed, the soil was washed with distilled water while sitting on a piece of filter paper in a funnel. The funnel was filled with distilled water and allowed to empty 5 times. Remaining soil was sent to Oklahoma State University (OSU) for percent SOM and nitrogen testing (Zhang and Wang, 2014). At OSU, each sample was weighed to 300mg in a foil cup. Then the samples were run through a Leco CN combustion analyzer, which gives the total carbon and nitrogen. The SOM was divided by 1.724 to find the organic carbon. The organic carbon was divided by the total nitrogen to find the C:N ratio.

Statistical Analysis

We used a two- way analysis of variance (ANOVA) with randomized block design to determine the main and interactive effects of plant cultivation type and soil depth to affect CO_2 evolution over time, cumulative CO_2 evolution, soil organic matter, and soil nitrogen dynamics. Datasets were tested for normality and homoscedasticity with the Shapiro-Wilk W-test and Levene test, respectively. Day 0 did not meet normality assumptions and was log (X+1) transformed before analysis. All analysis were done is JMP 12 (SAS Institute Inc., Cary, NC). The figures were created using SigmaPlot 12 (Systat Software, San Jose, CA).

Results

Cumulative CO_2 Evolution

Plant cultivation type had the strongest influence on cumulative CO_2 evolution ($F=4.31$ $p=0.02$; table 1). The perennial cultivation treatment had the greatest cumulative CO_2 evolution while the annual treatment had the least cumulative CO_2 evolution (Fig 1). Cumulative CO_2 evolution for the perennial treatment was on average 26.7% higher than the annual treatment and 10.9% higher than the prairie plots. Soil depth alone did not show a significant effect ($F=0.05$ $p=0.82$). The cultivation × soil depth interaction was marginally significant ($F=2.52$ $p=0.09$). It appears that there is strong cultivation treatment effect between the upper layers of the soil since the percent

increase for the upper soil depth between annual to perennial is 49%, while the annual cultivation treatment is only 9% lower than perennial for lower soil depth (Fig. 1).

CO_2 Evolution Over Time

Cultivation type and cultivation × soil depth were significant for CO_2 evolution on certain days throughout the experiment (Table 1). Conversely, CO_2 evolution was not influenced by soil depth over time ($p>0.05$ for all days). Initially, CO_2 evolution was mostly impacted by cultivation type (days 1, 3, 8, 15); annual and perennial treatments differed from each other, while the prairie treatment did not differ from either the annual or perennial. For example, CO_2 evolution in perennial soils was on average greater than in annual soils on days 1, 3, 8, 15, respectively by 61%, 36%, 55%, 45%. Cultivation × soil depth also initially had an interaction (day 1 to day 8). The upper soil depth in perennial treatment respired the most CO_2 overall (Fig 2). The prairie was intermediate in its CO_2 evolution. The percent increase from the annual to the perennial in the upper soil was 145%, 73%, and 113% on days 1, 5, and 8. The upper annual separates out from the other cultivations and depths with the lowest CO_2 evolution of all treatments.

Soil properties: soil organic matter and nitrogen

The total soil carbon and nitrogen responded to similar factors and in similar directions as the CO_2 evolution (Fig 3; Fig 4). Soil nitrogen was significantly different for each factor: cultivation type, soil depth, and cultivation × soil depth (Table, $p<0.05$). The largest effect was noted between the annual upper and perennial upper soil, with a 40% increase from annual to perennial treatments. SOM was only significant for cultivation type ($F=4.32$ $p=0.02$) and almost significant for cultivation × soil depth ($F=3.03$ $p=0.06$), but not influenced by soil depth. Both nitrogen and SOM identified the perennial and annual treatments as being different, while the prairie was intermediate. The perennial had about 20% more SOM than the annual. The prairie was almost exactly in the middle, being about 10% greater than the annual and 10% lower than the perennial. The C:N ratio was not significantly different between treatments or depths ($p>0.05$). All treatments had a mean C:N ratio of approximately 8:1.

Discussion

Plant cultivation type was the main driver of soil CO_2 evolution both over time and cumulatively. The perennial grain cultivation type exhibited greater soil CO_2 evolution relative to annual grain, but neither differed significantly from restored prairie. Further, perennial grain cultivation had greater total soil carbon, nitrogen, and SOM when compared to the annual; again, neither differed from restored prairie. Surprisingly, soil depth alone did not affect soil CO_2 evolution or soil C, N, and SOM. Instead, soil depth effects were contingent upon cultivation type. Perennial cultivation exhibited greater soil CO_2 evolution than annual cultivation at shallower depths than at deeper soil depths.

The perennial fields follow trends previously documented in the literature when comparing perennial fields to annual croplands, where the perennial fields have greater soil carbon and SOM (Wang *et al*., 2008, Wesemael *et at*., 2010, Glover *et al*., 2012). For instance, a meta-analysis by Guo and Gifford (2002) found that when sites were converted from pastures to croplands, fields lost on average 59% of their carbon stocks. Conversely, when croplands were converted to pastures, the fields had a mean 19% increase to the carbon stock. When comparing grasslands to croplands that have both been harvested for 75 years, grasslands had a percent increase of around 40% in the SOM (Culmen *et al*. 2010). Perennial grain crops are replanted every 3-5 years, which leads to less soil disturbance. Since the soil is held intact, it loses less carbon than annual crops to erosion and runoff (Gyssels *et al*., 2005, Cox *et al*., 2006). This longer life cycle also allows perennial grasses to develop deeper roots, which provides them with the ability to sequester more carbon in the soil (Glover *et al.* 2007).

Reference prairie, like the perennial cultivation treatment, was expected to have greater soil carbon than the annual treatment, but it was intermediate between the perennial and annual treatments and did not differ significantly from either one. In perennial monocultures, soil carbon can be stored in above- and belowground plant pools (shoots and roots respectively) instead of only in the soil pools, which was found to be the case when fields were used to grow the perennials alfalfa and meadow fescue grass, when compared to annual barley (Paustian *et al.,* 1990). The prairie has only been restored for 13 years. This may not be enough time for the

prairie to reach optimal functioning. For example, a tallgrass prairie that was restored 65 years prior still had 37% less soil carbon in the upper 25cm of soil when compared to a remnant prairie (Kucharik *et al.*, 2006). The prairie may have been also limited by soil nitrogen. Hunt *et al*. (1988) discovered that plant production in prairies was limited by nitrogen and significantly increased with fertilizer addition. Matamala *et al*. (2008) found evidence that soil carbon and soil nitrogen are tightly linked. While the amount of SOC did increase in their prairie soil with increased prairie age, C:N ratio did not change, indicating that the nitrogen increases in proportion to carbon. Our prairie C:N ratio was rather low at about 8:1, which may indicate that the prairie is more carbon limited than nitrogen limited. To grow new plant material the C:N ratio needs to be somewhere between 30-35; anything less will reduce will reduce production (Parton *et al*., 1988). The C:N ratio was similar for all the plant cultivation treatments even though total soil nitrogen and carbon varied significantly between treatments, which may suggest that carbon and nitrogen are closely linked in each of the treatments.

Similar to soil carbon, soil nitrogen was highest in the perennial treatment and lowest in the annual. Perennial grasses are known to slow down the nitrogen cycle when compared to annual cropping. This slowed nitrogen cycle leads to a reduction in the amount of nitrogen lost (Jackson, 2017). Annual grasses return less nitrogen (27ppm) to the soil than perennial grasses (37ppm) in part because the annual grass does less nitrogen mineralization throughout the growing season in a Mediterranean grassland system (Joffre, 1989). When comparing an annual corn-corn-soybean cropping to perennial grass (Switchgrass *Panicum virgatum*) the annual treatments did exhibit higher soil nitrogen mineralization and nitrification due to fertilization additions, but overall, lost considerably more soil nitrogen than the perennial grasses through nitrate leeching (Smith *et al.*, 2013). The corn also had much more nitrogen in its aboveground biomass than the switchgrass grass since the nitrogen is removed from the grass's aboveground biomass and returned to the soil at senescence. Paustain *et al.* (1990) discovered nitrogen mineralization to be higher in perennial grass (21g m^{-2}) than annual barley (9g m^{-2}). The perennial grass also had smaller carbon to nitrogen ratio, which indicated that more of its nitrogen was being released per unit of mineralized SOM.

Tilling disrupts the top 10cm of the surface soil of the annual fields. Tilling greatly impacts SOM and soil quality in agricultural ecosystems by altering the microbial biomass (Sapkota, 2012). Tilling rearranges surface soil with lower layers, allowing SOM to be eroded and accessible to microbes. It also causes microbes to become more concentrated in the surface layers (Smith and Paustain *et al*., 1990). Dou *et al*. (2008) observed that the soil depth from 0-15cm experiences the greatest disturbance in terms of microbial biomass, nitrogen, and carbon, while soil depths below 15cm do not appear to be strongly affected. Microbes in the upper layers are able to decompose the carbon from plant residues more quickly and release the carbon to the atmosphere as CO_2 (Bronich and Lal, 2005). Carter (1986) found that even shallow tillage reduced microbial biomass carbon and nitrogen by 10-23% when compared to no till.

Loss of SOC can also lead to a decrease in microbes since they use labile carbon as an energy source and building block for biomass (Holye *et al*., 2011). A reduction in microbes, such as nitrogen fixing bacteria, can disrupt nutrient cycling, leading to reduction in soil nitrogen (Holye *et al*., 2011; Kibblewhite *et al*., 2008). The annual treatment is tilled more often than perennial treatment, while the prairie is not tilled at all. Since they require less tilling, the perennials may help maintain microbial populations and nutrient cycling. When cultivation × soil depth was significant, carbon and nitrogen were always in the upper layer of soil (0-15cm) between the perennial and annual treatments, which is most likely caused by tilling. The nitrogen cycling exemplifies the difference tilling makes in nitrogen turnover, with the perennial treatment showing a 40% increase over the annual treatment in the upper layer.

Conclusions

We found the main and interactive effects of plant cultivation type and soil depth to affect soil C and N dynamics. First, we found that perennial crops promoted greater flux and storage of C and N than annual crops as, we initially predicted. Contrary to our predictions, the restored/reference prairie was intermediate between the annual and perennial treatments in respect to its carbon flux and C and N storage. Our findings indicate that perennials have a greater potential for carbon mineralization and sequestration than annual cultivation practices. Using perennial grains instead

of annual wheat had the potential to help maintain the soil health (carbon and nitrogen levels) while providing similar amounts of grain.

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Chapter 1: Tables and Figures

Table 1. Oklahoma regions (West, Central, East), collection site locations, latitude (degrees N), longitude (degrees W), mean annual precipitation, mean growing season temperature for the nine plant collection sites.

Gradient	Site	Latitude	Longitude	Precipitation (cm)	Temperature (°C)
West	Black Mesa State Park (BL)	36.665	102.046	43.56	27.7
West	Four Canyon Preserve (FO)	36.014	99.468	60.40	26.6
West	Packsaddle Wildlife Management Area (PA)	35.855	99.618	63.47	22.4
Central	Kessler Atmospheric and Ecological Field Station (KE)	34.985	97.528	109.09	22.4
Central	Chickasaw National Recration Area (CH)	34.504	96.95	102.34	23.8
Central	Tallgrass Prairie Preserve (TA)	36.846	96.423	104.06	22.4
East	John Nickle Preserve (JO)	35.915	94.973	119.43	22.8
East	Sequoyah National Wildlife Refuge (SE)	35.447	94.973	123.27	22.9
East	Fort Gibson Wildlife Management Area (FG)	35.798	95.251	118.29	23.1

Table 2. The average NEE and total biomass of all the mesocosms for each species. The Exotic species have greater biomass than the natives. The exotics also have a greater NEE average than the native species average. SE is the standard error.

Exotic Species	**Average NEE in µmols CO_2 m^{-2} s^{-1} (SE)**	**Average Total Biomass in g (SE)**
B. ischaemum	-3.95 (0.43)	19.75 (0.99)
S. halepense	-3.00 (0.35)	23.28 (1.46)
Exotic Averages	-3.47 (0.28)	21.52 (0.90)
Native Species		
S. nutans	-3.17 (0.34)	6.54 (0.57)
S. scorparium	-1.66 (0.27)	2.22 (0.33)
Native Averages	-2.41 (0.25)	4.38 (0.46)

Fig 1. The effect size representing the benefit associated with early arrival (as compared to arrival in the control group) of plant genotypes for each population (SS: *Schizachyrium scoparium*; SN: *Sorghastrum nutans*; BI: *Bothriochloa ischaemum*; SH: *Sorghum halepense*). Values are mean standardized effect size ±95% confidence interval.

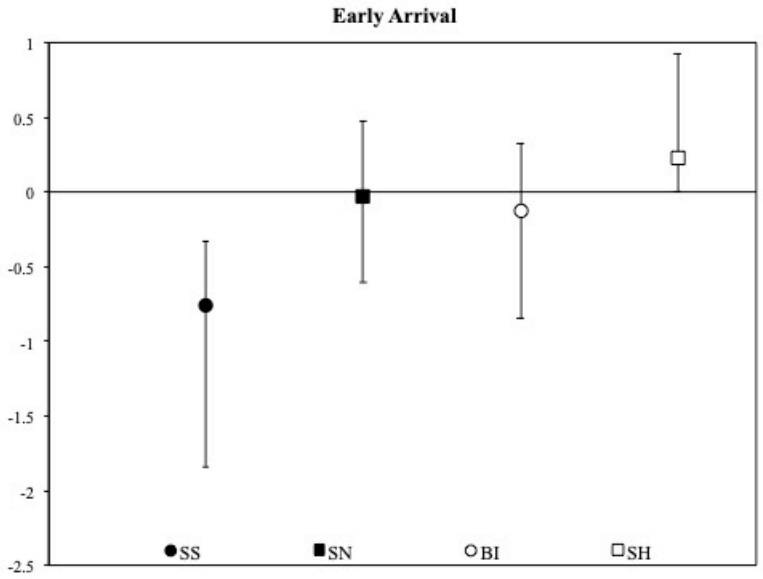


Fig 2. The effect size representing the cost associated with late arrival (as compared to arrival in the control group) of plant genotypes for each population (SS: *Schizachyrium scoparium*; SN: *Sorghastrum nutans*; BI: *Bothriochloa ischaemum*; SH: *Sorghum halepense*). Values are mean standardized effect size ±95% confidence interval.

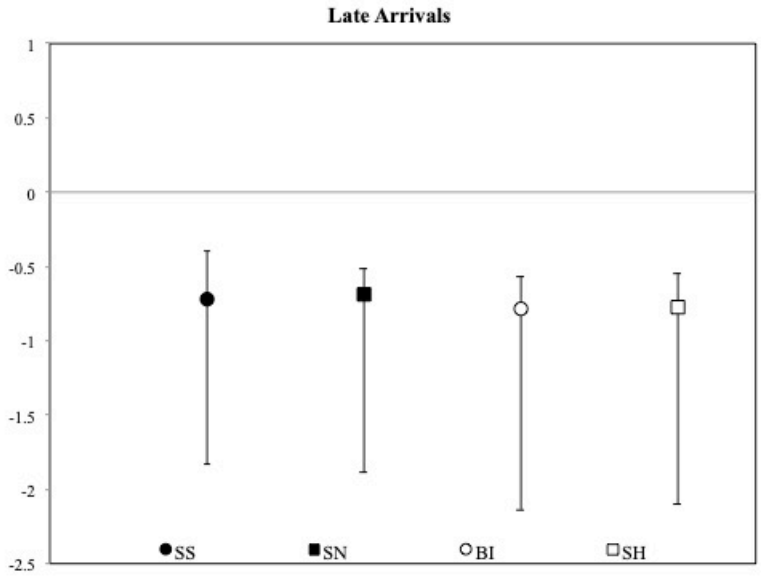


Fig 3. The relationship of phenotypic neighbor distance and biomass are not significant. (SS: *Schizachyrium scoparium*; SN: *Sorghastrum nutans*; BI: *Bothriochloa ischaemum*; SH: *Sorghum halepense*)

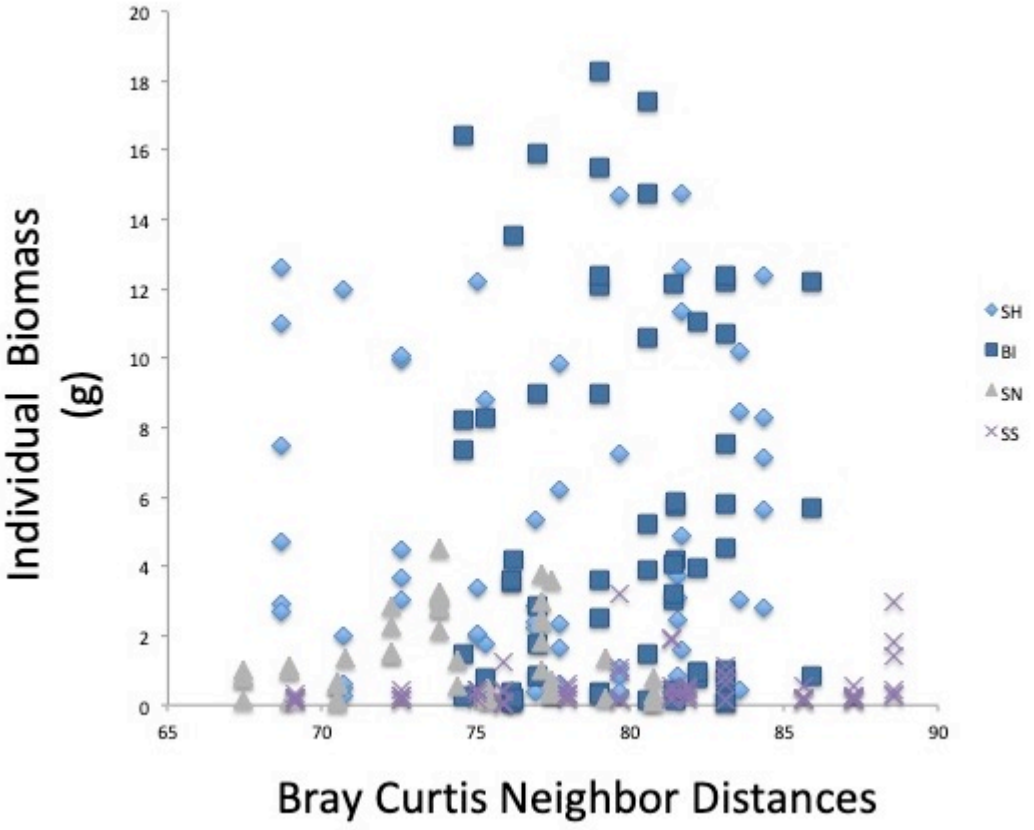
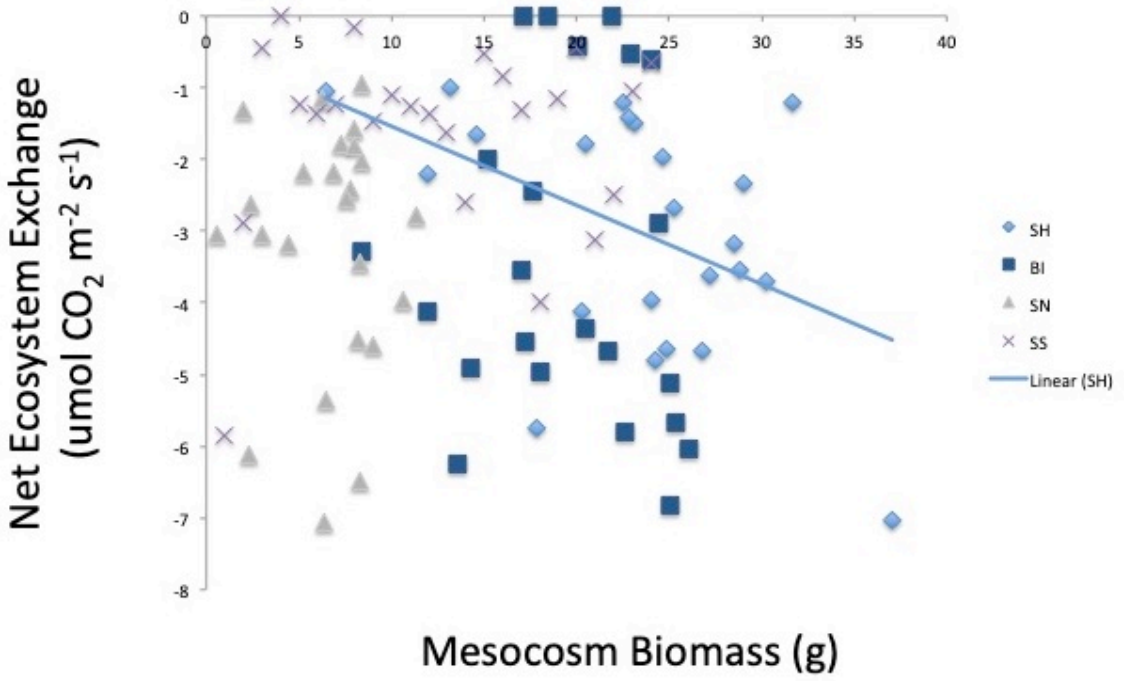


Fig 4. Regression of NEE and biomass for each species. Only *Sorghum halepense* had a significant relationship with biomass increasing with increased NEE. (SS: *Schizachyrium scoparium*; SN: *Sorghastrum nutans*; BI: *Bothriochloa ischaemum*; SH: *Sorghum halepense*)



Chapter 2: Tables and Figures

Table 1. Two-way ANOVA results testing for the main and interactive effects of cultivation type by soil depth both across time and cumulatively to affect soil CO2 evolution. F is the F statistic and p is the p-value. P-values with a "*" are significant.

Day	Cultivation Type: F	Cultivation Type: p	Soil Depth: F	Soil Depth: p	Cultivation* Soil Depth: F	Cultivation *Soil Depth: p
0	2.56	0.09	0.55	0.74	1.10	0.39
1	6.16	**<0.01***	0.15	0.70	3.52	**0.04***
2	0.64	0.53	2.05	0.15	0.22	0.80
3	3.53	**0.04***	1.33	0.26	1.33	0.27
5	1.41	0.26	2.70	0.06	3.60	**0.04***
8	5.73	**<0.01***	0.83	0.37	3.20	**0.05***
15	3.79	**0.03***	<0.01	0.95	2.20	0.12
30	2.21	0.12	<0.01	0.94	0.56	0.58
60	0.04	0.96	1.01	0.32	0.09	0.91
90	0.94	0.40	0.02	0.88	0.65	0.53
120	1.63	0.21	2.92	0.09	<0.01	1.00
Cumulative	4.31	**0.02***	0.05	0.82	2.52	0.09

Table 2. Two-way ANOVA results testing for the main and interactive effects of cultivation type by soil depth both across time and pace for nitrogen, SOM, and C:N ratio. F is the F statistic and p is the p-value. P-values with a "*" are significant.

Property	Cultivation Type: F	Cultivation Type: p	Soil Depth: F	Soil Depth: p	Cultivation* Soil Depth: F	Cultivation *Soil Depth: p
Nitrogen	8.16	**<0.01***	4.65	**0.04***	4.25	**0.02***
SOM	4.32	**0.02***	1.43	0.24	3.03	0.06
C:N ratio	1.79	0.18	2.95	0.09	0.42	0.66

Fig 1. The perennial treatment has significantly more CO_2 evolution than the annual treatment. Bars are means for vegetation cultivation treatments (annual, perennial, and prairie treatments) with standard error at upper and lower soil depths. Bars with different letters indicate a significant difference ($p<0.05$).

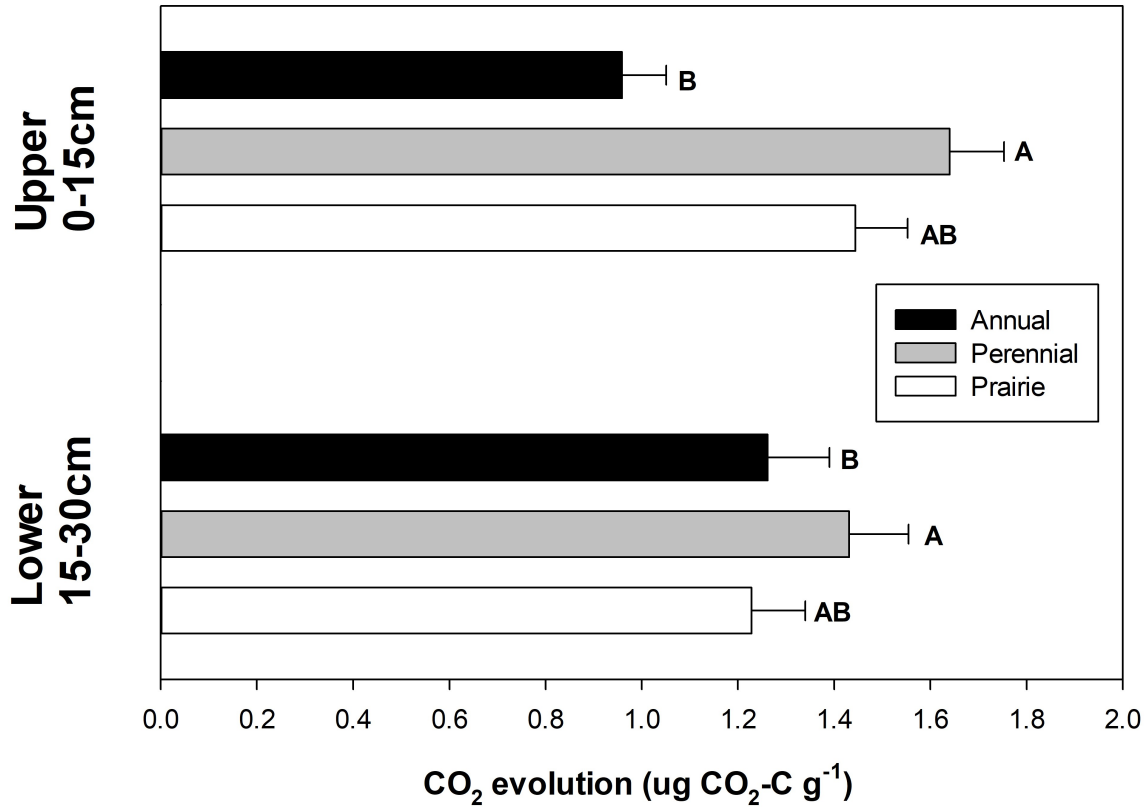


Fig 2. The CO_2 evolution over time for each treatment and depth. Each day is an addition of the CO_2 respired that day and all days prior. The annual upper has the least CO_2 evolution over time while the perennial upper has to most.

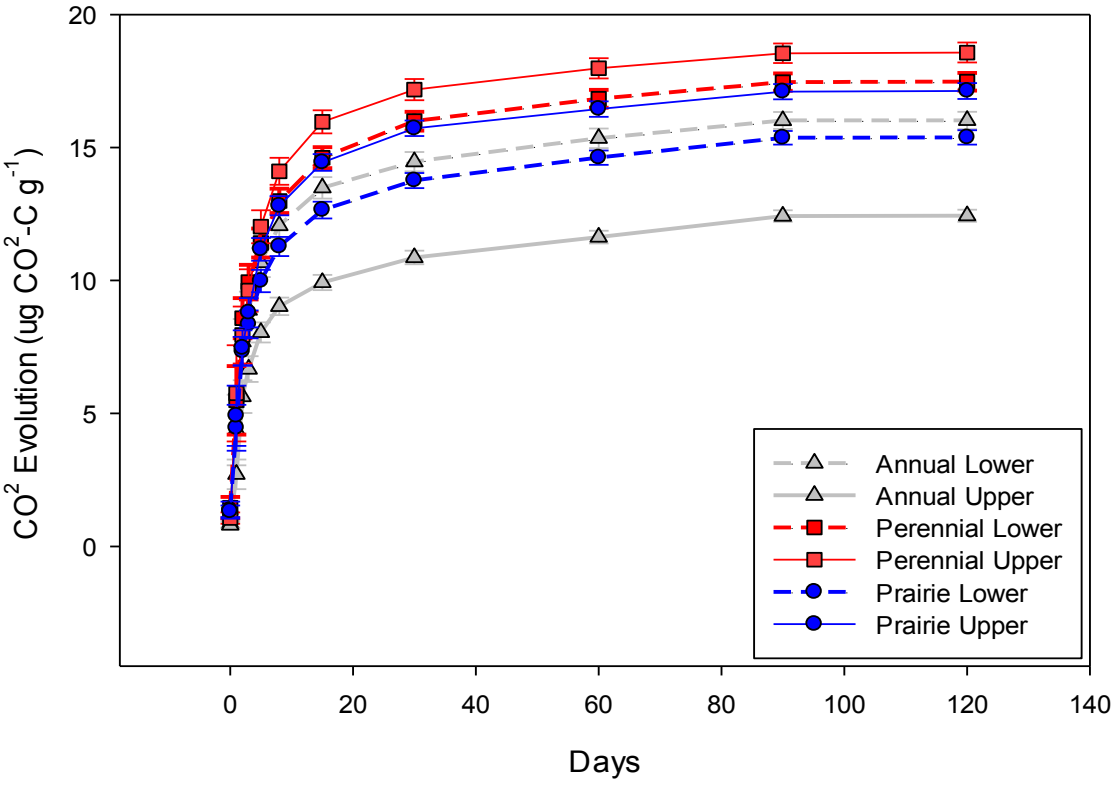


Fig 3. SOM in annually cultivated soils is significantly lower than perennial cultivated soils, but only at shallower depths. Bars are means for vegetation cultivation treatments (annual, perennial, and prairie treatments) with standard error at upper and lower soil depths. Bars with different letters indicate a significant difference ($p<0.05$).

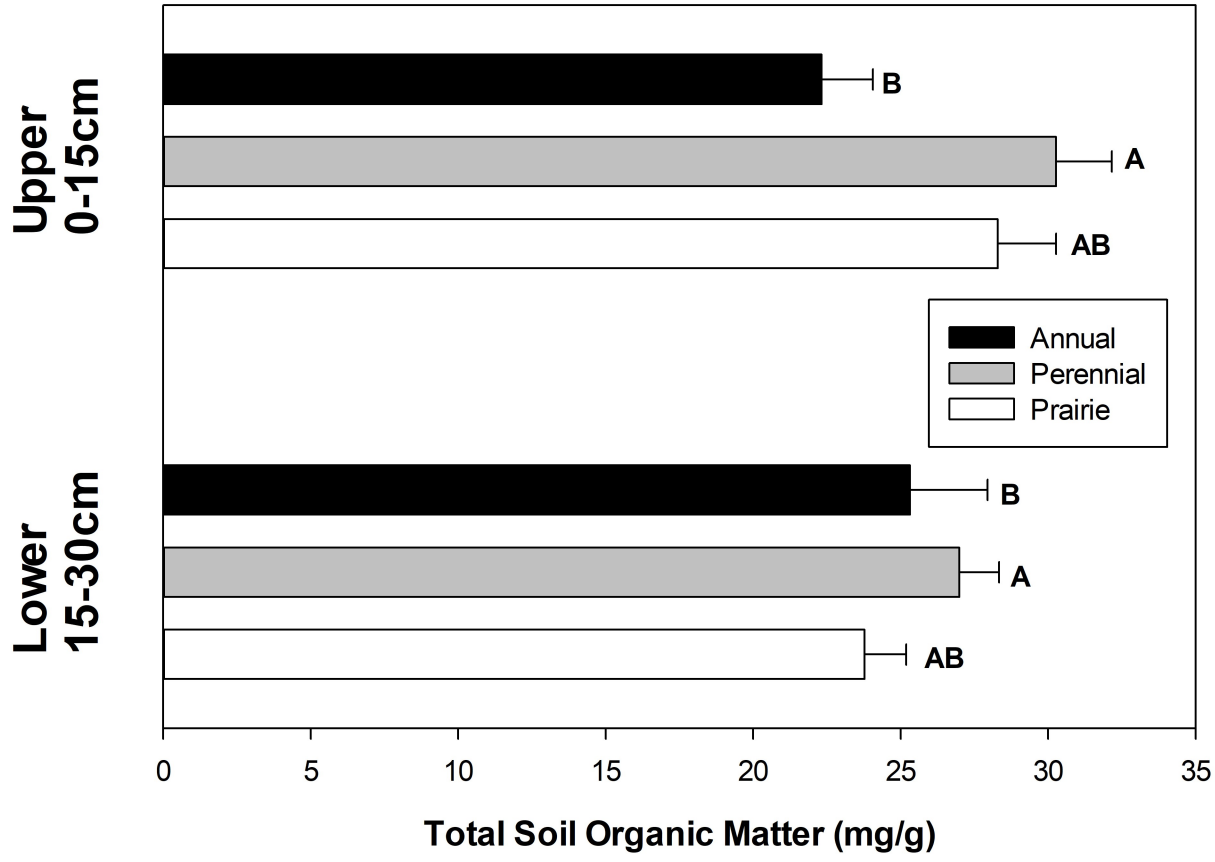
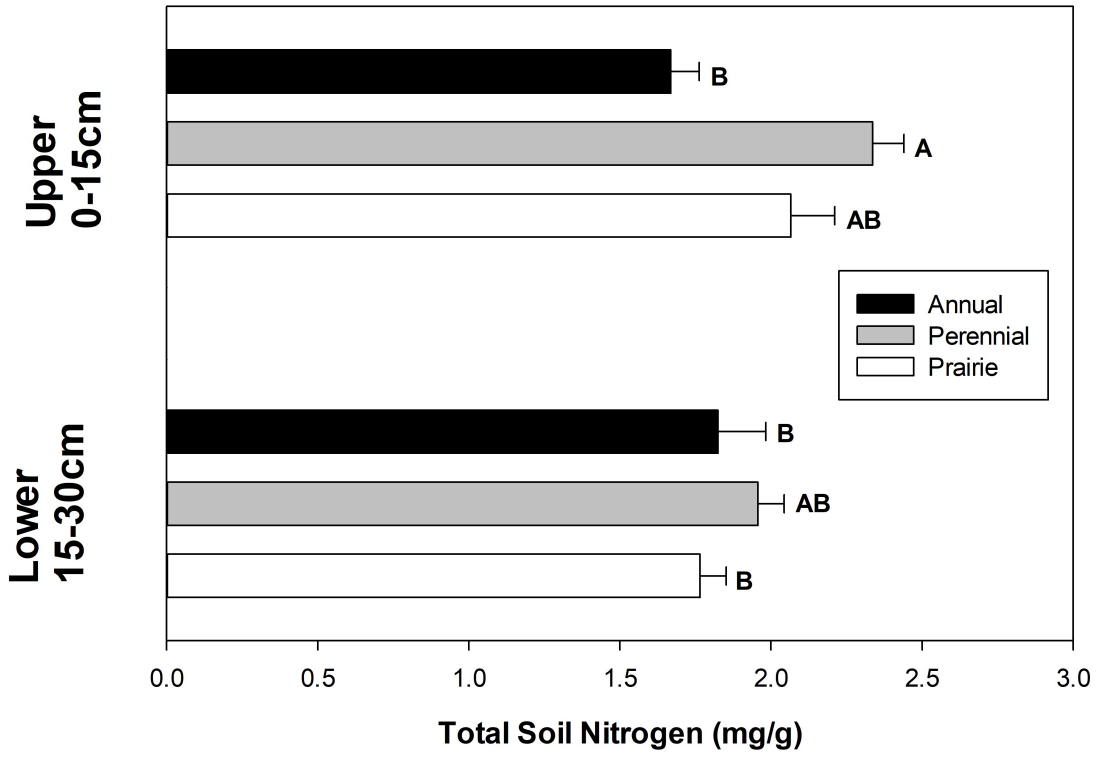
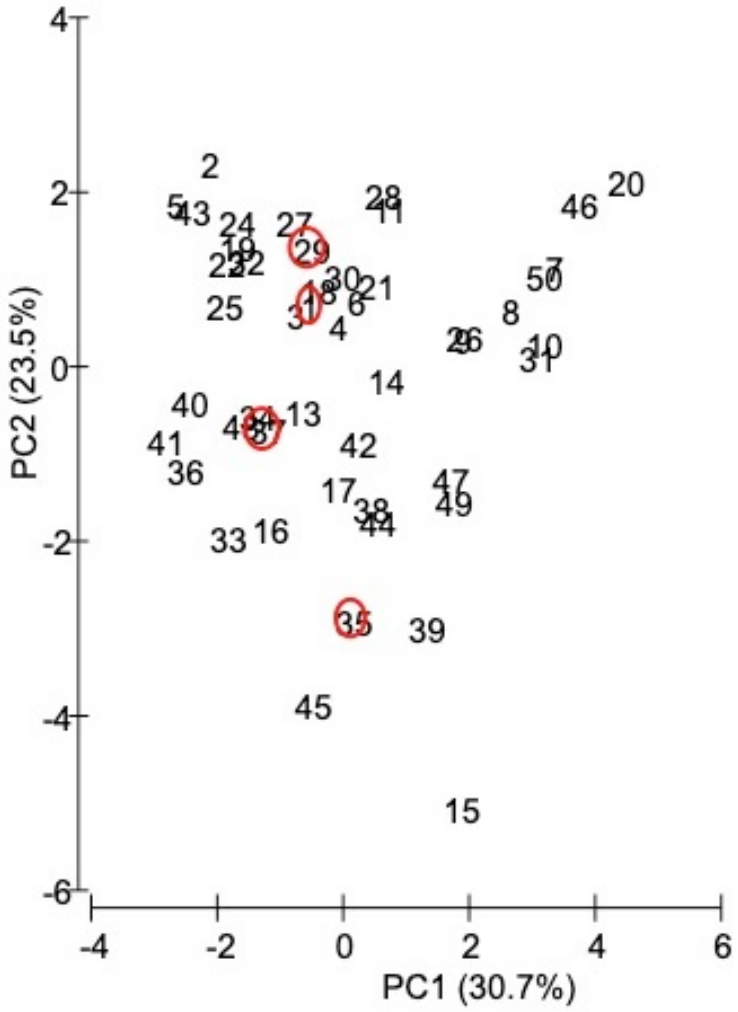


Fig 4. Total soil nitrogen in annually cultivated soils is significantly lower than perennial cultivated soils, but only at shallower depths. Bars are means for vegetation cultivation treatments (annual, perennial, and prairie treatments) with standard error at upper and lower soil depths. Bars with different letters indicate a significant difference ($p<0.05$).

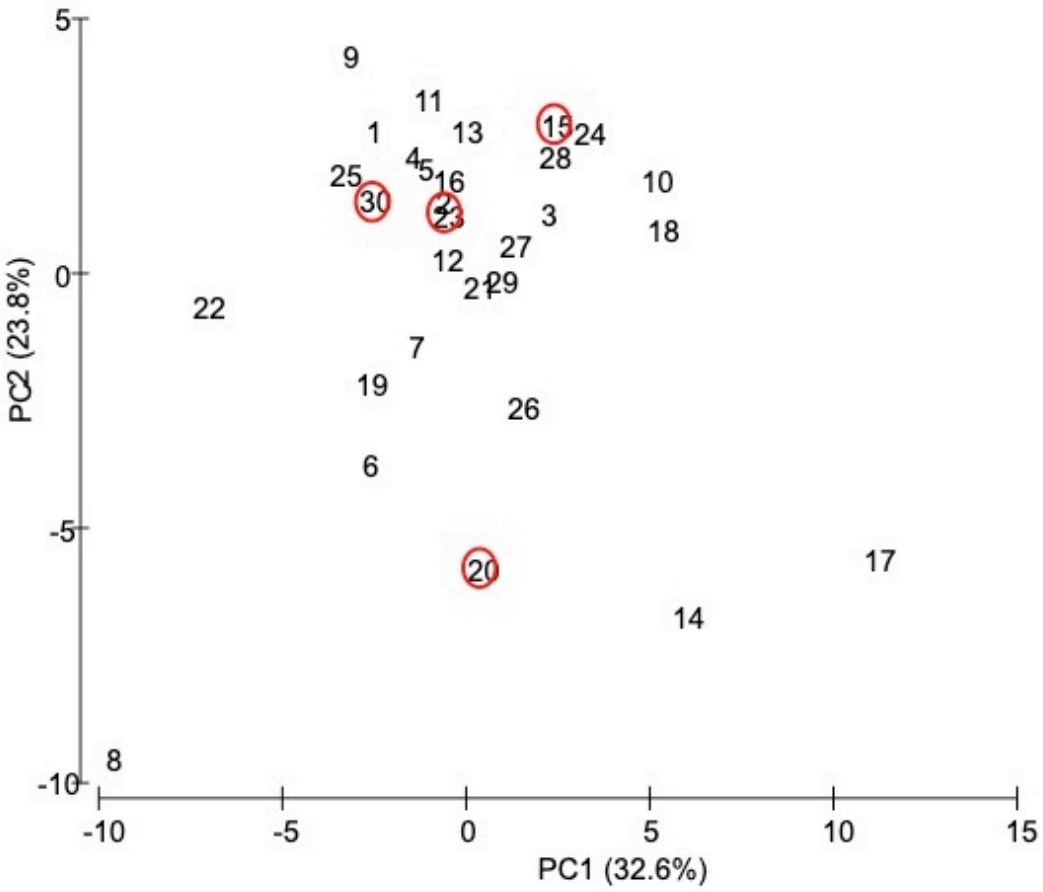


Appendix

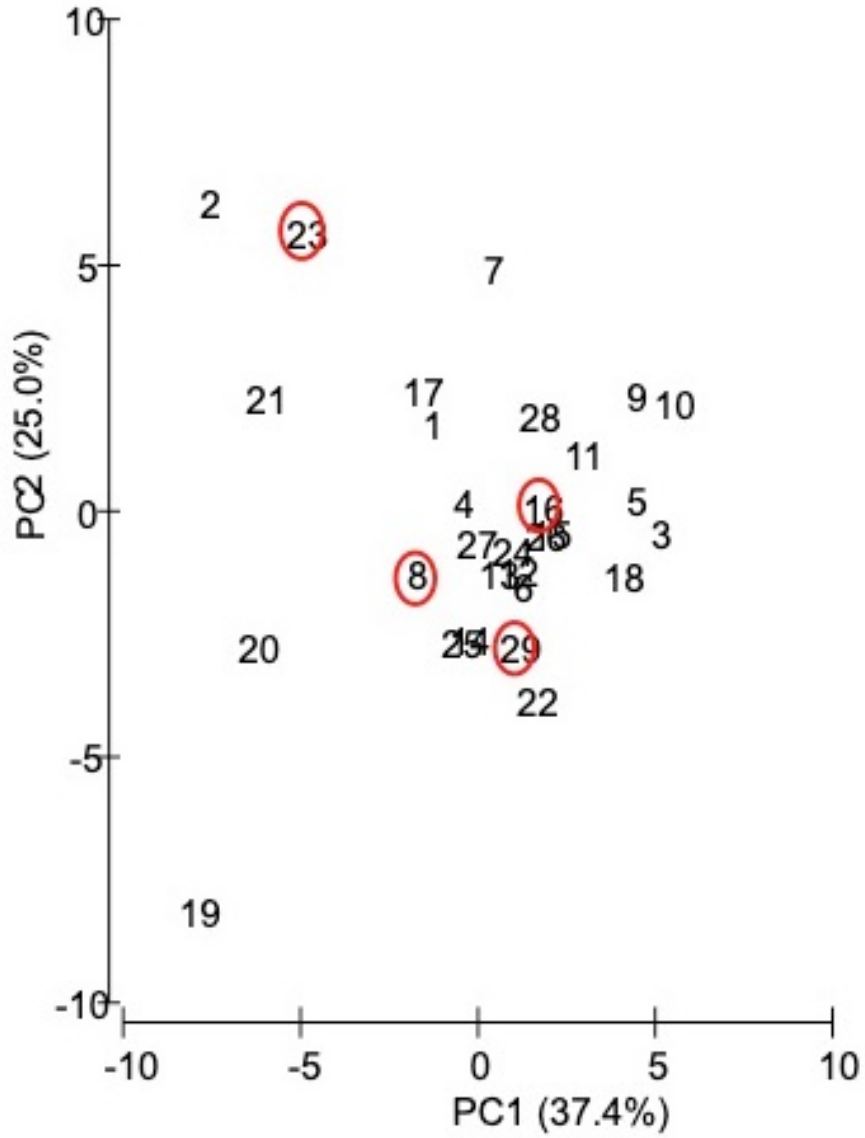
First two PC axes generated from a principal component analysis that included multivariate plant morphological (height), reproductive (inflorescence mass), physiological (specific leaf area) from *Schizachyrium scoparium* plant genotypes. In order to maximize trait variance, we selected four *Schizachyrium scoparium* with aim to maximize their separation across the x-axis (PC 1) and y-axis (PC 2). The selected genotypes included (PCA #: Site-Species-Individual Oklahoma region): 1:BL-SS-1 West, 29: KE-SS-1 Central, 35: PA-SS-3 West, 37: PA-SS-5 West. (Reference table 1 for sites.)



First two PC axes generated from a principal component analysis that included multivariate plant morphological (height), reproductive (inflorescence mass), physiological (specific leaf area) from *Sorghastrum nutans* plant genotypes. In order to maximize trait variance, we selected four *Sorghastrum nutans* with aim to maximize their separation across the x-axis (PC 1) and y-axis (PC 2). The selected genotypes included (PCA #: Site-Species-Individual Oklahoma region): 15: FO-SN-6 West, 20: KE-SN-3 Central, 23: 23: PA-SN-3 West, 30: PA-SN-6 West. (Reference table 1 for sites.)



First two PC axes generated from a principal component analysis that included multivariate plant morphological (height), reproductive (inflorescence mass), physiological (specific leaf area) from *Sorghum halepense* plant genotypes. In order to maximize trait variance, we selected four *Sorghum halepense* with aim to maximize their separation across the x-axis (PC 1) and y-axis (PC 2). The selected genotypes included (PCA #: Site-Species-Individual Oklahoma region): 8: FG-SH-2 East, 16: KE-SH-1 Central, 23: SE-SH-6 East, 29: TA-SH-6 Central. (Reference table 1 for sites.)



First two PC axes generated from a principal component analysis that included multivariate plant morphological (height), reproductive (inflorescence mass), physiological (specific leaf area) from *Bothriochloa ischaemum* plant genotypes. In order to maximize trait variance, we selected four *Bothriochloa ischaemum* with aim to maximize their separation across the x-axis (PC 1) and y-axis (PC 2). The selected genotypes included (PCA #: Site-Species-Individual Oklahoma region): 11: CH-BI-5 Central, 13: FO-BI-1 West, 21: KE-BI-5 Central, 29: TA-B-3 Central. (Reference table 1 for sites.)