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Targeted short-term nutrient reduction to manage Ventenata dubia an invasive winter annual grass: soil and plant responses

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Targeted Short-Term Nutrient Reduction to Manage *Ventenata dubia* an Invasive Winter Annual Grass: Soil and Plant Responses

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MASTER'S THESIS

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Targeted Short-Term Nutrient Reduction to Manage *Ventenata dubia*

an Invasive Winter Annual Grass: Soil and Plant Responses

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Invasive winter annual grasses, IWAGs, have degraded extensive ecosystems around the world and continue to invade new ones yearly. IWAGs readily form large monocultures or near monocultures, thus management and restoration goals largely focus on maintaining or increasing plant diversity in impacted ecosystems. Unfortunately, common management methods also reduce native plant diversity and harm the soil microbiome. These effects require additional measures to be taken, like reseeding, and plant diversity is still usually well below remnant targets. Early season short-term nutrient reduction to manage IWAGs is largely unexplored and would potentially decrease IWAG abundance, active earlier than most plants, but impact later season species less. Low rates of labile carbon, as sucrose, were applied to soils in a Pacific Northwest semi-arid grassland in early spring to stimulate microbial growth and temporarily reduce nutrient availability to the IWAG *Ventenata dubia*. Inorganic nitrogen was tracked throughout the experiment and plant and soil microbial community changes were determined at the end of the growing season. Labile carbon application reduced nitrogen at the beginning of the year, but effects did not persist to mid may when most plants were still active and soil moisture was not limiting. Treatments reduced *V.dubia* cover, per area seed production, and seed mass with no corresponding impact on perennial or other annual plants, except at the highest application rate when annual cover was reduced by 2%. The soil microbial community, determined via PLFA and NLFA analysis, was largely unchanged at the end of the season with slightly higher bacterial biomass and, importantly, no reduction in AMF abundance. These results suggest that this method has few negative impacts on the plant and soil community aside from a reduction in *V.dubia* cover and possibly its seed bank. This short-term nutrient reduction method has the potential to not only target IWAGs, active early, but also any non-native plants targeted for management that are active earlier or later than the native plant community.

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Introduction

Intact semi-arid grasslands are one of the most endangered ecosystems in the world (Ceballos et al. 2010). Their high fertility and moderate annual rainfall made them attractive for development into dryland agricultural and cattle grazing. The remaining intact grasslands have been further degraded by the introduction of exotic plants (Mack et al. 2000, Vitousek and Walker 1989). *Ventenata dubia,* North Africa grass, is an **invasive winter annual grass** (hereafter **IWAG**) that has become dominant in many Pacific Northwest semi-arid grasslands, where it is responsible for reduced biodiversity and decreased range and cropland value (Wallace & Prather 2015). In 2001 *V. dubia* was expanding its range by over 1.2 million hectares per year (Novak et al.. 2015) mostly in semi-arid grasslands. Since then, *V. dubia* has begun to readily invade sagebrush dominated arid systems, and regions with intact biological soil crusts (Norton et al. 2018).

The importance of the soil microbial community for the management of IWAGs and successful restoration of native plant species is becoming increasingly apparent. Native seed germination in converted agricultural soils is increased when the soil is inoculated with microbes from native areas (Hawkes & Hamman 2013). When arbuscular mycorrhizal fungi, AMF, abundance and

diversity is reduced, native plant abundance is also reduced, making room for other species (Wilson et al. 1999, Lilleskov et al. 2002). AMF facilitate a diverse plant community especially in cool season systems dominated by C3 grasses (McCormack et al. 2015) and an intact AMF community prohibits dominant plants form forming monocultures. Cheatgrass, a common IWAG, has lower shoot mass when grown with AMF while native perennial grasses like *Elymus elymoides*, squirrel tail, has the opposite response (Sieg et al. 2013). This further implies that soils with reduced AMF abundance may benefit IWAGs. Further, a naturally occurring gram negative bacteria *Pseudomonas flourescens* D7 and the fungi *Pyrenophora semeniperda,* black finger of death, have been proposed as methods to manage the IWAG cheatgrass. In many cases, when soils were inoculated with these microbes cheatgrass abundance decreases (Kennedy et al. 1991, Quinney et al. 2007).

Common methods of managing *V. dubia* and other IWAGs are prescribed fire, mowing, tilling, and herbicides. Each of these methods have limitations and most have negative effects on grassland plant and soil communities. Prescribed fire is used to remove the litter layer that can give IWAGs a competitive advantage over native plants (Nissen et al. 2017). Burning will also increase nutrient cycling and availability, conditions that favor IWAGs, and often result in elevated IWAG density in subsequent years (Davies et al. 2019). Mowing can be effective if the timing is appropriate, but if IWAGs have already produced seeds mowing can facilitate dispersal and invasive density later (Knochel et al. 2014). Uneven and rocky soils make mowing improbable as well as another common

management technique, tilling. Where possible, tilling can be used to bury the invasive plant seed bank and remove existing plants. This, of course, also eliminates the entire plant community and seed bank including any desirable species. Tilling is an extreme disturbance event and disturbances largely benefit IWAGs more than native perennial species. If land is not treated and seeded properly before and after tilling, invasive annuals will often become dominant (Beard et al. 2007). Beyond the impact to the plant community, the soil community is also disturbed by tilling. AMF are much reduced in land that has been tilled. Non-AMF, decomposer, fungi hyphae are severed as well and most fungi in general have reduced abundance (Miller et al. 2004).

Various herbicides are common components of most IWAG management plans. IWAGs germinate in the Fall or early Spring and are active in times when most native plants are dormant, especially early spring. The general approach is to apply herbicides pre- or post-emergence in the Fall or early in the Spring when it is thought that only IWAGs will be harmed by herbicide application. IWAG reduction the first year following herbicide application can be quite dramatic and, in some cases, nearly complete (Morris et al. 2016). An experiment in Northern Idaho semi-arid grass and croplands found up to 99% control of *V. dubia* in the first year with fall herbicide application. In this case, the herbicide caused limited harm to the existing perennial plants, although perennial abundance did not increase either (Wallace et al. 2015). However, the following year, *V. dubia* abundances rebounded to levels at or above those of control plots. Wallace et al. (2015) did not report any plant community data besides *V. dubia* abundance in

year two so perennial response after the first year is unknown. In some cases, reduced IWAG abundance can persist for multiple years, especially when coupled with thatch removal and other control efforts (Morris et al. 2016). The reduction of IWAGs in year one via herbicide application followed by a complete recovery of IWAG abundance, with little or no effect seen after multiple years, is a common theme in IWAG management research (Rudd & Elseroad 2011, Monaco et al. 2009). This suggests that single herbicide applications alone are unlikely to result in a long-term reduction of *V. dubia*.

Considering that in many cases invasive cover can rebound to levels above those in control treatments, it appears that herbicides may be causing some change in the plant or soil community that benefits IWAGs in subsequent years. A long-lasting reduction in IWAG abundances has been exhibited by reducing or eliminating the IWAG seed bank. Researchers in a Colorado temperate grassland were able to eliminate cheatgrass by applying herbicide for 4-5 years consecutively, depleting the seed bank entirely (Nissen et al. 2017). Unfortunately, it also drastically altered the plant community. The study showed that the plant community shifted from cold season dominated to warm season dominated species. Unfortunately, warm season grasses are not well represented in Inland Northwest grasslands leaving some uncertainty as to how our grassland community would respond. Managing invasive plants via herbicides can alter the plant community in several ways including reducing diversity, as low abundance plants are lost and plants active early in the year are reduced. (Willis et al. 2002, Nagel et al. 2012).

Herbicide use can also have harmful effects on the soil microbial community. Glyphosate application reduces the viability of AMF spores and root colonization at almost any application rate (Druille et al. 2013, 2016). Repeated glyphosate applications alter the soil microbial composition increasing microbes, often fungi, adapted to metabolizing glyphosate (Lorenz et al. 2012). Herbicides can also decrease the ratio of bacteria to fungi in the soil which may indicate more dramatic changes to the soil community (Busse et al.. 2006). Even a single application of glyphosate and other herbicides at recommended rates can reduce the abundance of gram-negative bacteria, including many plant beneficial pseudomonas species and some that act as natural IWAG biocontrol agents (Kennedy et al. 2014, Kennedy 2018).

Considering the potential negative impacts to the native plant and soil community from the more common methods of managing invasive winter annual grasses, it is important to continue exploring alternative and sustainable methods. One method of managing IWAGs is by reducing inorganic soil nutrients such as ammonium and nitrate. Invasive plants (often early successional) generally perform better than natives (often late successional) in systems with high nutrient availability and cycling (Tilman & Lehman 2001, Prober 2016). In fact, invasive plants often cause high nutrient conditions via increased disturbance and other plant soil feedbacks (Gibbons 2017). Thus, it is thought that reducing nutrient availability decreases invasive plant fitness and may increase native perennial fitness (Flory et al. 2012, Oliveira & Eller 2017). Nutrients available to plants can be reduced by adding a source of carbon to the

soil (Figure 1). In many cases, the soil microbial community is limited not by nutrients, like plants, but by sources of energy, carbon (Tateno et al. 2011). Thus, when organic matter is decomposed by soil microbes, excess nitrogen is excreted in inorganic forms that are then available for plant use (Scheu & Tiunov 2004). Also, as the soil fungi and bacteria are consumed by higher trophic levels, often nematodes and other invertebrates with a higher carbon to nitrogen (C: N) ratio, additional amounts of inorganic nitrogen are secreted (Culman et al. 2004). Adding a source of carbon to the soil with a high C:N ratio increases the abundance of many microbes by increasing carbon availability. Microbes then become more nutrient limited and begin to compete with plants for inorganic nutrients, reducing the amount available for plant use (Lamb 1980). This nutrient reduction effect can also be impacted by the presence and abundance of higher trophic levels, like nematodes, that increase nutrient availability. These higher trophic levels generally have a larger C:N ratio than their food source, bacteria and fungi, resulting in elevated nutrient mineralization and availability. As their prey increases the abundance of these higher trophic levels may also increase causing increased nutrient availability and less of a reduction effect or perhaps a shorter duration of nutrient reduction (Figure 2).

Either recalcitrant or labile forms of carbon can be added to the soil to reduce inorganic nutrient availability. Recalcitrant forms include lignin and cellulose, e.g. sawdust, which are difficult for microbes to access, cause a long-term nutrient reduction, but is slow to take affect (Wanek et al. 2014, Livne-Luzon et al. 2016, Kadlec 2016). Labile forms, like sucrose and glucose, are easily

assimilated forms of carbon and cause a rapid nutrient reduction, but the effect does not last long unless labile carbon is reapplied (Jones 2014, Funakawa et al. 2015, Lunt 2002, Jackson and Bleier 2007). In many cases both types of carbon have been used in conjunction to get both quick, labile, and long-lasting, recalcitrant, nutrient reduction.

Most previous research has focused on a long-term nutrient reduction by adding recalcitrant and/or labile carbon to benefit late successional native plants. Unfortunately, results are inconsistent and often do not last. In some cases, invasive species are reduced, and native species increased (Blumenthal et al. 2003, de Barse and Morris 2013, Burke et al. 2013). In others, both invasive and native plant species are reduced, or no effect is seen (D'Antonio & Corbin 2004, Szitar et al. 2014). These contradictory results may be a result of different field and soil conditions or they may be due to an incorrect assumption that perennial plants are less effected by low nutrient conditions than invasive plants. In fact, it appears that many annuals outperform perennials in low nutrient conditions since they do not allocate as many resources to non-reproductive tissue production (Sieg 2012). Perennial species still experience the low nutrient condition and IWAGs still experience a priority effect benefit, giving them a net advantage (Drenovsky et al. 2011).

Herbicide application targeted at the early season growth habit of IWAGs is a cornerstone of common management methods. Unfortunately, there have been few studies testing the efficacy of nutrient reduction via labile carbon application in a similar manner. Applying labile carbon once early in the year may

temporarily reduce nutrient availability when *V. dubia* is solely active with a reduced impact on late season species. In this way, I may get more consistent results than experiments that concentrated on long term nutrient reduction. The very few studies that have tested this method were largely not explicitly designed to do so. When a single application of labile carbon has been used to reduce invasive perennial grass abundance in a temperate grassland, a reduction was seen in greenhouse studies but not field studies (Bakker and Mitchell 2011). This is likely because reduced nutrient availability had faded by the time the target invasive had become active or the effect was similar in non-target perennial species as well. When IWAGs have been the target of early season labile carbon addition, most studies see a reduction of IWAG abundance or productivity in the first year. Beckstead (2003) found that when areas with high cheatgrass cover, >85%, were treated with 21 g carbon (C) m^{-2}, cheatgrass density was reduced but biomass was largely unchanged. When cheatgrass density is high, competition for resources from other plants will be low, allowing cheatgrass to access nutrients when the effect of carbon had worn off. Unfortunately, areas with lower cheatgrass abundance, and likely higher plant diversity and competition, did not receive labile carbon treatments in this study (Beckstead and Augsburger 2003). Beckstead (2003) did not monitor seed production but, in many cases, when density is reduced, seed production per plant increases resulting in no decrease in total seed production (Mack& Rice 1991). Steers et al. (2011) used targeted early sucrose application to reduce nutrients when IWAGs were active while not impacting other native annuals that germinate later in their

Southern California arid creosote grassland (Steers et al. 2011). They applied 240 and 120 g C m^{-1} the first year. These values were targeted to add 100:1 and 50:1 ratios of labile carbon to soil inorganic nitrogen and treatments were applied for three years. They observed a reduction in IWAGs relative abundance the first year, but this did not persist to year three. This customized method has been relatively unexplored and has the potential to have a proportionally larger effect on IWAGs than late season plants. Unfortunately, the Steers study took place in an annual dominated system so treatment effects would likely reduce most other species equally.

The high cost of labile carbon has been the largest detractor for using this method to manage IWAGs. Chambers et al. (2011) saw a reduction in cheatgrass abundance at an application rate of 150 g C m^{-2}. In a later study, the lowest recommended effective application rate to reduce medusahead was 64 g C m^{-2} (Pyke 2010). With a current cost of labile C, as sucrose, of approximately \$0.86 kg^{-1}, these treatments would cost approximately \$2700 ha^{-1}, and \$1170 ha^{-1} with only a partial reduction in IWAG abundance for one year. Both studies initially applied herbicides that removed the existing established plant community, including any establish perennial or late season species. This resulted in an artificial annual dominated ecosystem, though Chambers et al. (2011) did include perennial seed application as a treatment. While these pre-treatments may characterize some systems undergoing restoration and may reduce confounding field effects, it also reduced established plant competition for resources later in

the season, instead depending on seedling survival and not an existing diverse plant community.

An important aspect of utilizing temporally targeted and short-term soil reduction to manage IWAGs is the determining the duration of the nutrient reduction. The effect needs to wear off, allowing nutrients to become increasingly available, before the end of the growing season so that late season species will not be negatively impacted alongside the IWAGs being targeted. Aside from Steers et al. (2011) none of the other studies have tracked inorganic nitrogen or other nutrients often enough, or at all, through the growing season to determine if or when nutrient availability rebounded to control levels. Steers et al. (2011) applied relatively high treatments in a nutrient poor system and as a result nitrogen did not recover to control levels before the end of the growing season. In most other studies, that tracked inorganic nitrogen, resin capsules were used and collected infrequently. This method causes less disturbance to the system but can only show season long changes in soil nutrients so are largely not beneficial in determining the duration of the nutrient reduction effect (Chambers et al. 2011, Pyke et al. 2010). Jackson and Bleier (2007) performed a glasshouse experiment analyzing the duration of nutrient reduction from labile carbon application, at 420 g C m^{-2}. They saw that inorganic nitrogen (N) was reduced through week 6 post application and the effect did not persist to week 24. Unfortunately, they did not measure N in the intervening weeks so could not determine approximately when nitrogen became available again.

In addition to the duration of the nutrient reduction it is also important to understand how the soil microbial community is responding to labile carbon treatments. To my knowledge, the studies that have tested nutrient reduction to manage invasive plants have largely neglected treatment effects on the soil microbial community (Beckstead and Augsburger 2004, D'Antonio and Corbin 2004, Bakker and Mitchell 2011, McLendon and Redente 1992). At most they monitored total microbial biomass via extractable microbial nitrogen or carbon measurements. These are, at best, coarse grained and can only show changes in total microbial abundance and not changes in microbial community characteristics (Steers et al. 2011, Pyke et al. 2010, Szitar et al. 2014). Steers et al. (2011) tracked microbial N monthly throughout the growing season and saw alternating periods of both higher and lower microbial biomass compared to controls with no consistent trend. Interestingly, at the end of the growing season high treatments had both significantly lower microbial and inorganic N than controls, when an inverse relationship is expected. Pyke et al. (2010) monitored both microbial N and C and saw the expected inverse relationship during the growing season, higher microbial N and lower inorganic N, with an increasing effect at higher sucrose application rates, though with no difference from control by July. Szitar et al. (2014) found consistently higher microbial C in plots that received sawdust treatments but likewise did not track any changes in soil microbial groups. Higher trophic levels in the soil microbial community have also been completely ignored in nutrient reduction studies and this could lead to reduced effects or inconsistent results.

Overall, the objective of this research is to determine if labile carbon can serve as an effective means to reduce the dominance of *V. dubia* and tilt the soil ecosystem in favor of native species. I further sought to understand how labile carbon alters the soil microbiome. Specifically, I tested the following predictions: 1) Applying small amounts of labile carbon, C, early in the growing season (when the IWAG is one of the few species active) can temporarily reduce nutrient availability, with a larger effect with more C. 2) These lower application rates will allow soil nutrients to recover to control levels by mid-season when soil moisture is not yet limiting for most species. 3) Early season targeted nutrient reduction will effectively reduce the IWAG *V. dubia* cover and per area seed production (seed bank). 4) Perennial species will benefit from decreased competition with microbes and lower *V. dubia* abundance later in the season and will increase plant diversity. 5) Since many annuals are active around the same time as *V. dubia*, these other annual plants will also be reduced by treatments but to a lesser degree. 6) Considering my prediction that nutrient reduction effects will not persist throughout the growing season, I do not expect large treatment effects on the soil microbial community will be seen at the end of the year. 7) More specifically, total microbial biomass will be increased at successively higher treatment rates (especially non-AMF), AMF abundance will not be reduced, and nematode abundances will be elevated due to an increased prey source,

Methods

Study Site

My study area is a 100-hectare portion of the Stubblefield area of Turnbull National Wildlife Refuge (TNWR) in Eastern Washington (Figure 3) 47.404927N, -117.528282E. Mima mounds are the primary land type and consist of unstratified accumulations of glacial loess up to 25 m in diameter and 2 m in height (Allen & Cox 1987). Mounds are separated by intermound areas characterized by generally shallower soils with soil depths ranging from 1 to 100 cm. Intermound soils often have abundant biological soil crusts and lower late season soil moisture. Precipitation in the study year was largely representative of the standard climatic conditions with a few exceptions. March precipitation was 80% of normal and April was 60% higher than average, and June only received 44% of normal rainfall.

Study Design

Ten experimental blocks, with a minimum area of 13 m^2, were interspersed across intermounds throughout the study area at least 100 m apart in locations that have extensive and relatively homogenous *V. dubia* abundance. Each experimental block was further subdivided into seven 1 m^2 plots. Soil depths ranged from 10-40 cm among experimental blocks but were less variable among plots in each block. In late March 2018, timed to coincide with *V. dubia's* most rapid growth, each plot received one of seven sucrose treatments; no addition (control), low, moderate, or high using a C3 sugar or low, moderate, or high using a C4 sugar (Table 1). These treatment levels corresponding to 0:1,

10:1 (50 g), 20:1 (100 g), and 50:1 (250 g) carbon (as sucrose) to inorganic nitrogen ratios, respectively. Mean inorganic nitrogen abundance used for calculations were approximately 8 μg g^{-1} dry soil from samples collected in the same area in 2012 (Anicito 2013). Using both C3 and C4 derived sucrose, which are different from the local soil carbon sources, allowed me to determine how the added carbon changes the soil community and identify what groups are actively assimilating the carbon (Table 1).

Field Methods

Soil respiration was measured weekly throughout the growing season using a Li-Cor 6400-xt infrared gas analyzer (IRGA) on permanently installed soil collars. Soil collars, 10.5 cm diameter, were installed to a uniform depth in four plots per experimental block at the beginning of the season. Soil collars were placed in every control plot in the experiment and alternating blocks had collars in C3 or C4 sucrose treatments. Measurements were taken for each block (n=40) at approximately the same time of day each week to reduce daily diurnal and temperature variation that may have occurred.

Soils were collected in the top 10 cm from every plot (n=70) every three weeks throughout the 15-week growing season, for a total of 5 sampling periods, using a 2.5 cm diameter soil corer. The soil corer was sterilized between plots using a 70% methanol solution and allowed to dry. During each sampling period four 10 cm deep soil cores were collected from each plot, one from each corner, to get a better whole plot representative sample. Soil was placed in individual

plastic zip bags and then in a cooler while sampling continued. Soil samples were brought back to the lab, sieved to 2 mm, and divided in half. Samples to be used for phospholipid fatty acid (PLFA), neutral lipid fatty acid (NLFA), and inorganic nitrogen analysis were frozen at -80°C within 5 hours of sample collection. The other portion were stored at 4°C for nematode extraction and analysis, soil moisture determination, and organic matter content.

To survey plant species composition, percent cover was visually estimated for each plant species in 1 m^2 plots in late June 2018. The two tallest inflorescences, of the five most ubiquitous species (*V. dubia, Poa bulbosa, Poa secunda, Acmispon americanus,* and *Bromus japonicus*) were collected from each quadrant of every plot, a total of eight inflorescences per species, to compare seed counts and average seed mass.

Lab Methods

I extracted nematodes from 10-20 g field moist soil (n=70) for each sampling period over 48 hours using Baerman funnels. Functional feeding group composition and total counts were performed using a compound microscope (Yeates et al. 1993). The resulting extracted nematodes were stored at 4°C until counts could be performed within 1-3 weeks. Every nematode counted was identified to functional group. Extracted nematodes were then stored at -80°C. Approximately 5 g of field moist soil were dried at 70°C to constant mass in order to determine gravimetric soil moisture. Nematode abundances per gram dry soil.

This dried soil was further analyzed for organic matter content via loss on ignition (LOI) at 450°C for 4 hours.

A portion of the soil stored at -80°C was air dried in an over at 35°C for three days. Inorganic nitrogen was determined using the 2M KCl method (Maynard 1993). Approximately 5 g of air-dried soil was extracted for 30 minutes using 35 mL of 2M KCl and filtered through 0.45 um EZFlow Syringe Filters with Foxx nylon membranes. The extracted solution was analyzed for both nitrate (NO_3^-), and ammonium (NH_4^+) via the cadmium reduction and TKN and ammonia gas diffusion method, respectively, using an Alpkem 3 flow analyzer (OIA 2009a, b, c, OIA2009 b). A portion of the 2M KCl extractant was used for pH determination using an Oakton pH6 Acorn Series pH meter.

Between 15 and 30 mg of soils collected from each block before treatment application (n=10), from each plot three weeks post application (n=70), and from each plot fifteen weeks post application (n=70) were also analyzed for $\delta\,^{13}C$ and total N at the Cornell University Stable Isotope Laboratory (COIL). Soil samples were analyzed via a ThermoFinnigan Delta Plus mass spectrometer and a NC2500 elemental analyzer. Two replicates of each sucrose treatment type, C3 and C4 derived sucrose, were sent to UC Davis Stable Isotope Facility for $\delta^{13}C$ analysis. Isotope data are reported in delta notation compared to Vienna Pee Dee Belemnite (VPDB):

$$\delta^{13}C = (({}^{13}C/{}^{12}C_{sample}) / ({}^{13}C/{}^{12}C_{VPDB})) * 1000\ ‰$$

Soil microbial communities were analyzed using soil lipid assays. All glassware was acid washed overnight and then burnt in a muffle furnace at

450°C for 4 hours to remove all lipids. Volumetric glassware and caps were only acid washed. Phospholipids are susceptible to oxidation due to light and the presence of oxygen, so all extracted samples are stored under argon and dried down under argon and/or vacuum. All solvents used were of HPLC grade.

Lipids were extracted using a method derived from Bligh and Dyer modified by Frostegård et al. (Bligh and Dyer 1959, Frostegård et al. 1991). Approximately 5 g of field moist soil stored at -80°C were placed in 50 mL glass centrifuge tubes. A recovery standard phospholipid, PC(13:0/13:0), was added to each sample and blank. Each sample was extracted twice for two hours using a sequential application of buffered citric acid, chloroform, and methanol at a 0.8:1:2 ratio. After centrifugation the supernatant from each extraction was decanted and combined into new centrifuge tubes. Additional citric acid and chloroform were added and allowed to separate undisturbed overnight in the dark, to avoid oxidation. The next day the upper aqueous layer is removed and the lower organic (chloroform) layer dried down in vacuum centrifuge evaporator with a solvent trap. Samples are covered with argon and stored at -20°C until separation.

Separation of lipids into neutral, glyco, and phospholipids is done using silicic acid chromatography using 500 mg 10 mL silica columns. Neutral lipids are eluted and collected using 4.5 ml of chloroform. Glycolipids are eluted using 5 mL acetone and this fraction is discarded. Phospholipids are eluted using 5 mL of methanol and are collected.

Neutral and phospholipids are converted to fatty acid methyl esters (FAMEs) via mild alkaline methylation using a 0.2M KOH methanol solution for 30 minutes in a 37°C water bath. Fames are extracted by washing three times with 2 ml hexane.

FAME samples were analyzed using an Agilent 7820A GC coupled to an Agilent 5975 MS using a 30 m HP-5MS (5%-Phenyl)-methylpolysiloxane column (250 µm ID x 0.25 µm film thickness) and He carrier gas. The system ran in split-splitless mode, 25:1 ratio, and constant flow of 1 ml/min. One 2 µl injection was analyzed at an initial temperature of 160°C followed by a 3°C increase per minute to 190°C, held for 15 minutes and then increased 10°C per minute to 255°C for a total run time of 31.5 minutes. Fatty acid methyl ester (FAME) abundances were determined by quantitation against the hexaethylbenzene internal standard. Individual lipids were identified using a NIST database and comparison to elution orders in the current literature. Further lipid identification was also performed at the UC Davis Stable Isotope Facility.

Standard FAME naming conventions are followed where the first number indicates the number of carbon atoms followed by a colon and a number indicating the number of double bonds. If a double bond is present the bond location is identified by a ‘w’ and a number indicating the position from the aliphatic end. The prefixes *cyc, Me,* and *OH* indicate cyclopropyl, methyl, and hydroxy groups respectively while *i* and *ai* prefixes indicate iso and anteiso terminal branching. Gram positive bacteria abundances were indicated by iso and anteiso terminally branched and mid chain branched FAMEs. Actinomycetes

bacteria, a subset of gram positive, are indicated by FAMEs with a mid-chain branch at position 10. Gram negative bacterial abundances were indicated by FAMEs with an unsaturation at w7 or w5 and cyclopropyl FAMES. Non-AMF fungi were indicated by 18:2w6 neutral and phospholipids. Arbuscular mycorrhizal fungi were indicated by 16:1w5 neutral and phospholipids.

A subset of both the neutral lipid and phospholipid derived FAMEs were sent to UC Davis Stable Isotope Facility for CSIA using a GC-C-IRMS. Four neutral and phospholipids from the high C3 and C4 sucrose treatments (n=16) were analyzed for $\delta^{13}C$ to determine the fate of added labile carbon. CSIA of FAMEs can be used to determine the $\delta^{13}C$ of individual lipids in a complex mixture like soil lipid extractions.

Statistical Analysis

The effect of sucrose treatment on plant community (percent cover) and microbial community (PLFA and NLFA assays) was analyzed in R statistical software using lme in the nlme package (R Core Team 2019). Linear mixed effects models were utilized using experimental block as a random factor. Each block had an individually weighted variance, calculated with the Satterthwaite approximation, which does not assume equal variance among blocks. The effect of sucrose treatments and time since treatment on nematode abundances, inorganic nitrogen abundance (ammonium and nitrate), organic matter, soil respiration, and soil moisture were also analyzed using linear mixed effects models using sampling period (week) and treatment as fixed effects and block as

a random factor, again using the Satterthwaite approximation to calculate variance. Tukey's HSD tests were performed on mixed model results using the emmeans package with treatment, and week when appropriate, as fixed factors and block as a random effect. Estimated marginal means and standard errors from the emmeans analysis are used for graphical representations of model results.

Changes in plant and microbial communities were each analyzed separately via permanova in Primer 7 and PERMANOVA+ using sucrose treatment as a fixed effect and experimental block as a random factor. Plant and microbial abundances were square root transformed to reduce the impact of high abundance species before the resemblance matrix was calculated using Bray-Curtis similarity. Plant and microbial communities were further analyzed for overdispersion in both sucrose treatment and block using the Permdisp function in Primer 7 and PERMANOVA+ (Clarke and Gorley 2015). Non-metric multidimensional scaling, NMDS, was also performed in R using the Vegan package using default settings and 1000 permutations to graphically show the difference between experimental blocks as well as the effect of treatment on the plant and microbial community composition. Plant communities and soil lipid profiles for the NMDS were square root transformed and standardized using Wisconsin double standardization (R Core Team 2019).

Results

Ammonium, NH_4^+, was reduced by sucrose treatments in the first six weeks of the experiment ($p=0.0079$). Three weeks post application control plots had NH_4^+ concentrations 19.4, 23.9, and 31.8% higher than low, moderate, and high treatments respectively (Figure 4, Table 2). In week 6, the effect size was more variable but low, moderate, and high treatments were still 23.2, 28.2, and 39.4% lower than controls, respectively. The treatment effect was not apparent after week 6 and NH_4^+ availability continued to decrease to 1.46 ppm by week 15. Nitrate data for week 6 were lost due to instrument error so only weeks 3, 9, and 15 are shown. In week 3, nitrate was lowest in high treatments followed by low, moderate, and finally control plots (Figure 5, Table 3). Weeks 9 and 15, showed slight non-significant decreased nitrate abundance in both moderate and high treatments.

Plant surveys identified thirty different species across the study area, twelve of which were annual. Annual species dominated, on average, with over 68% coverage, but most of this, 57%, was due to non-native species. Perennial cover, 32%, was represented nearly equally by both native and non-native species. Grasses were the most common plant type, 72% of total cover, but were dominated by non-native species while forbs, 28% of total cover were dominated by native species, 22%. Species richness varied from just 7 in some plots up to 14, with no treatment effects on either richness or diversity (Table 5). Plant community composition was affected by both sucrose treatments and random block effects, as shown by significant Permanova results (Table 4). The NMDS

ordination of the plant community illustrates that the variation between experimental blocks largely overshadows individual treatment effects (Figure 6).

Ventenata dubia cover varied from 3% to 75% with most plots having at least 50% cover. Compared to control plots, sucrose treatments reduced *V. dubia* abundance; with greater decreases with increasing sucrose application rates (Figure 7, Table 5). Low, moderate and high sucrose treatments resulted in 8, 25, and 34% reductions in *V. dubia,* respectively. Abundance of other annual plant species was also reduced by 2.1% compared to controls, but only by high sucrose treatments. Sucrose treatments did not impact perennial species or other non-native species cover at any application rate. Native plant cover increased significantly in all treatments, compared to controls, by an average of 3.9% ($p<0.006$).

V. dubia seed abundance was unchanged by sucrose treatments but average seed mass was significantly reduced by 7.8, 11.1, and 15.3% at low, moderate, and high treatment levels, respectively (Figure 8, Table 6). Sucrose treatments increased seed abundance of the nitrogen fixing native annual, *A. americanus,* from 3.9 seeds per inflorescence in control plots to an average of 4.5 seeds in treated plots ($p=0.0014$). *P. secunda (*native*), B. japonicas* (nonnative)*,* and *P. bulbosa* (nonnative) seed abundance and mass were unchanged by treatments (Figure 9, Table 7).

The PLFA assay identified 31 individual phospholipids which were used to compare the soil microbial community. Permanova results of the PLFA lipid profile indicate little impact of sucrose treatments; like vegetation communities,

block affects were more related to variations in the soil microbial composition (Table 4. Figure 10). More specifically, total PLFA was higher in all treatments but was only significantly higher in the low treatment plots by 11% (Figure 11, Table 9). Total NLFA concentrations were non-significantly different across treatments with a slight decreasing trend from control to high treatments. The total NLFA to total PLFA ratio was similar for control, low, and moderate treatments but was significantly, 21.8%, lower in the high treatment.

Gram positive indicator PLFAs were also significantly higher than control in the low treatment, 11% higher, with now difference between controls and either moderate or high treatments. Gram negative indicators followed the same trend but both low and moderate treatments were higher than controls. Cyclopropyl to gram negative ratios, often used as an indicator of environmental stress or low carbon availability, were unchanged by treatments. Actinomycetes, a type of gram-negative bacteria able to consume chitin, were likewise apparently unaffected by treatments (Figure 12, Table 9).

Non-AMF fungi PLFA were relatively unchanged by treatments but high application plots were significantly lower than plots that received a moderate rate (Figure 13, Table 8). Non-AMF fungi NLFA nor the ratio of NLFA:PLFA were affected by treatments (Figure 14, Table 8).

AMF PLFAs showed a similar trend to total PLFA abundance with low treatments having the highest abundance and control the least, but treatment effects were not significant (Figure 13, Table 8). There was not a significant treatment effect on AMF NLFA abundances though high treatments did have

slightly lower abundance than controls. The AMF NLFA: PLFA ratio was not different in any treatment compared to controls but high application rates were significantly lower than both low and moderate treatments by 12.9 and 9% respectively (Figure 13, Table 8).

The bacteria PLFA to fungi PLFA ratio was likewise relatively unchanged by treatment when compared to controls but high treatments had a significantly larger ratio than low application treatments and a were marginally higher than plots with moderate treatment rates. The bacterial PLFA:fungal NLFA ratio increased from control to high treatments but only had a significant difference in high treatments compared to controls. High treatments also had a larger bacterial PLFA: fungal NLFA ratio than low treatments as well (Figure 14, Table 7)..

Compound specific $\delta^{13}C$ were analyzed in C3 and C4 derived high sucrose treatments for both PLFA and NLFA fractions in four of my experimental blocks. The PLFA fraction showed that gram negative bacteria, gram positive bacteria, and non-AMF fungi assimilated a significant portion of the sucrose added in these treatments (Figure 16, Table 9). The PLFA isotope ratios also suggest that AMF may also have assimilated a portion of the treatments, but effects were only marginally significant. The was no significant differences in isotope ratios in either the AMF or non-AMF NLFA fractions.

Nematode abundances generally increased throughout the growing season regardless of treatment level ($p<0.0001$). Total nematode abundance nearly tripled throughout the season, increasing from 1.69 per gram dry soil in week 3, to 4.67 per gram by week 15. Bacterivores, fungivores, root/fungal

feeding, and omnivores followed the same seasonal trend with no significant treatment effects. Moderate and high treatments tended to have higher, non-significant, total nematode abundances than control plots until week 15 when abundances were more similar. The only nematode feeding group that showed a significant treatment effect were the plant parasite group that had lower abundances in week 12 and 15 in treatment plots compared to controls ($p=0.022$). In week 12 low and moderate treatments contained 41 and 47% fewer plant parasites per gram than control plots, respectively. In week 15 plant parasites were 43% lower in moderate treatment plots compared to controls (Figure 17, Table 10).

Soil organic matter ranged in experimental blocks from just 1.5% to over 9%. Organic matter was not impacted by sucrose treatments but did increase throughout the growing season, increasing from an average of 4.4% in week 3 to 5.2% at week 15. Soil respiration ranged from 0.32 to 10.9 g $C/m^2/sec$ throughout the growing season. Most of this variation was due to differences in experimental block, individual plot, and time period with no significant effect of sucrose treatments (Table 11). Treatment did not generally have a significant effect on soil moisture throughout the growing season. High treatments did have slightly higher moisture throughout the experiment compared to controls and at week 12 post application moisture was significantly high in the high treatment compared to controls by 2.1% (Figure 18, Table 11).

Discussion

My study demonstrates that sucrose application is an effective method of nutrient reduction, even at relatively low application rates compared to other studies. I further showed that carefully timed nutrient reduction can reduce abundance of the IWAG, *V. dubia* over the short-term, without negatively affecting perennial plant species or, except at the highest application rate, other annual plants in my system. This targeted low application method had limited effects on the soil microbial community by the end of the growing season with total microbial biomass was only slightly elevated, largely as a result of increased gram-negative and gram-positive bacteria, while fungal groups, including the important AMF, showed little to no change. Nematode abundances were also largely unchanged by these low application rates indicating that subsequent year treatments will likely impact nutrient availability similarly. Increased bacterial and fungal consumers in subsequent years could decrease nutrient reduction effects by increasing mineralization rates.

Other studies have had similar results, but both my low and moderate treatment rates are well below experimentally optimized rates determined by others (Chambers et al 2011, Pyke et al 2010). I attribute the similar results, but at lower application rates, to the diverse intact plant community I retained. These established plants, often active later in the season, may benefit from increased late season nutrient availability and could then compete with *V. dubia*. A 30% reduction in *V. dubia* is not comparable to first year reductions that can be seen by other common control methods, like herbicide, that will often see over 90%

control the first year from a single application (Morris et al 2016, Wallace et al 2015). This short-term nutrient reduction control method differs from others in two major ways. First, herbicides often significantly reduce biodiversity and can cause local extinction of rare plants (Willis et al 2002, Nagel et al 2012, Nissen et al 2017). This short-term nutrient reduction method did not impact any diversity measures or non-IWAG seed characteristics implying that future diversity should also not be impacted. Second, when IWAG density is reduced by standard control measures, including herbicide, per plant seed production is often higher due to increased nutrient and moisture availability and reduced competition (Mack & Rice 1991). This effectively causes no net reduction in the IWAG per area seed production and the future seed bank, often resulting in a complete recovery of IWAG cover in subsequent years (Rudd & Elseroad 2011, Morris et al 2009). Targeted short-term nutrient reduction not only decreased *V. dubia* cover it decreased *V. dubia* seed mass and kept per plant seed production constant. This should result in reduced per area seed production and the IWAG seed bank. Taken together, these results could have long term effects on the abundance of *V. dubia*. Especially with annually repeated labile carbon applications.

While *V. dubia* abundance and seed characteristics were decreased by our nutrient reduction experiment, other plant groups were less impacted. Standard IWAG management methods, like herbicide application, will often exhibit limited or no negative impacts on perennial species but annual species are often reduced, being active around the same time as IWAGs (Willis et al.

2002, Nagel et al. 2012, Wallace et al. 2015). Impacts of this short-term nutrient reduction method on perennial species were like herbicide research, no harm done, but impacts on the annual community were much less. As a result, this short-term nutrient reduction method may be effective in systems with desirable annuals even if they are active within a few weeks of the IWAG being targeted.

Impacts of common IWAG management methods on the soil microbial community are often negative especially on the AMF community where they can dramatically reduce the number of viable spores and disrupt the hyphal networks AMF rely on for nutrients and other resource acquisition (Druille et al. 2013, 2016, Kennedy et al. 2014). AMF are important in restoration for both maintaining and increasing plant diversity as well as decreasing invasive plant abundance (Bever et al. 2017, Wilson et al. 1999, Lilleskov et al. 2002, Sieg et al. 2013). When these AMF networks are harmed by standard restoration efforts, we may be providing IWAGs and other non-native plants with the conditions needed to thrive, which is counterproductive at best. Considering that this targeted short-term nutrient reduction method was able to reduce the abundance of the IWAG *V.dubia* but did not appear to harm the AMF community the soil microbial community may be in a better condition to maintain plant diversity in the future compared to when standard management techniques are used.

Common management methods, tilling and herbicide especially, often negatively impact the rest of the soil microbial community as well, not just AMF (Miller et al. 2004, Kennedy et al. 2014). It is likely this harm done to the soil microbial community is part of the reason that many management and restoration

projects are unsuccessful, or unsatisfactory. Considering this, I wanted to make sure that this short-term nutrient reduction wasn't causing long-term effects to the soil microbial community. Results from compound specific isotope analysis (CSIA) of PLFA and NLFA fractions indicate that all microbial groups, aside from AMF, appear to be utilizing the labile carbon applications to increase biomass. Both multivariate analysis of the soil lipid profile and analysis of individual groups via PLFA and extracted nematodes, showed that treatment effects by the end of the growing season were limited. This suggests that short-term nutrient reduction as a potential IWAG management technique should not have long term negative impacts on the soil microbial community that would give IWAGs a net benefit, e.g. reduced biodiversity. Other indicators of microbial stress including fungal NLFA to PLFA ratios and cyclopropyl PLFAs to gram-negative bacteria lipids were also largely unchanged. Further evidence that the soil microbial community is largely unharmed by this proposed IWAG management technique.

Prior nutrient reduction efforts to manage invasive plants have almost entirely ignored the diversity of the soil microbial community and have discounted the importance of higher trophic levels or neglected them and their importance in nutrient availability entirely (Blumenthal et al. 2003, de Barse and Morris 2013, Burke et al. 2013, D'Antonio & Corbin 2004, Szitar et al. 2014). This may partial explain why they have had inconsistent results. The few short-term nutrient reduction studies performed have, likewise, neglected the multitrophic structure of the soil and may be an additional reason why they required higher application rates before significant IWAG reductions were observed, compared to my study

(Pyke et al. 2010, Chambers et al. 2011). When labile carbon treatments have been applied for multiple years, a reduced effect on the plant community can sometimes be seen (Steers et al. 2011). It is possible that high labile carbon application rates could be increasing the abundance of higher trophic levels causing a reduced effect in subsequent years. Though, since the soil community was not analyzed in these studies, it is unclear if this is the case. In either case, the abundance of higher trophic levels that consume soil bacteria and fungi, nematodes in this study, appear to be unaffected by applying low rates early in the growing season in a temperate semi-arid grassland. This suggests that treatment effects in subsequent years should be relatively predictable due to similar predation on fungi and bacteria and thus consistent mineralization rates.

This targeted short-term nutrient reduction method shows a lot of potential to be able to reduce the abundance of invasive winter annual grasses while limiting negative impacts on both perennial and annual establish species. There do not appear to be any negative impacts on the soil microbial community, especially regarding AMF abundance and higher trophic levels consumers. This study was only conducted for one season, so it is difficult and inappropriate to draw large conclusions from it thus far. Also, plot sizes were relatively small, 1 m^2, so edge effects were large especially regarding plant and fungal effects that have structures that likely extended outside of experimental plots. Beyond that, while precipitation and temperature were close to averages during the experimental year it is unclear how annual variations in precipitation and soil moisture could alter treatment effects.

There are many questions that will need to be answered by future research before this methods viability can be determined. Larger experimental plots should be utilized to minimize edge effects and the experimental area should be extended to include arid systems, in addition to the semi-arid ecosystems in this study, since both are being impacted by IWAGs. It is unclear how much of the effect on IWAG abundance is in fact due to nutrient reduction and how much may be due to osmotic stress because of the dissolved labile carbon. More extensive and multiple year testing needs to be completed to determine how long-term applications impact the plant community and ideal treatment duration. Understanding how historic contingencies impact treatment effects needs to be understood, including historic usage, soil moisture, annual precipitation timing, and temperature at time of application to name a few. While this targeted short term, nutrient reduction does not appear to have extensive impacts on the soil microbial community by the end of the first growing season, these effects need to be monitored and analysis needs to be expanded to take a closer look at how microbial species composition changes as well. Lastly, while I targeted the short-term nutrient reduction at IWAGs, active early in the year, there is potential for this method to target any plant that has a life history strategy different from the other species in the system. For example, Reynecke (2015) saw a significant reduction in perennial grass abundance the following year when labile carbon was applied in early summer with no change in annual species abundance.

Conclusion

By applying low amounts of labile carbon early in the growing year to a system with an intact plant community with species active early and late in the season I was able to see significant reductions in the IWAG *V. dubia* at rates below similar research. Competition for nutrients was increased early in the year from increased microbial abundance and later by an intact plant community. This two-stage competition strategy did not increase per plant IWAG seed production, thus reducing the seed bank. Plant diversity, perennial, and annual cover and seed characteristics were largely unharmed by this method, with only a slight reduction of annual cover at the highest application rate. Likewise, the soil microbial community was unharmed as well. In fact, bacteria were increased in most treatment rates by the end of the season and, importantly, AMF abundance and energy conditions were unchanged by treatments. This suggests that the soil microbial community is intact and should be in a condition conducive for future management success and increased plant diversity.

The costs of this management technique are relatively high compared to herbicide use. Herbicide costs do vary but can be as little as $20 per acre. By applying treatments to an intact plant community, I was able to reduce the effective labile carbon application rate by 33% compared to most previous research. The cost of the moderate labile treatment rate can be as low as $220 per acre depending on carbon source, which is still significant compared to herbicide costs. Also, like herbicide use, a single application is unlikely to cause a significant reduction in IWAG abundance in the long term, thus treatments will

need to be applied for multiple years. Cost savings, compared to standard restoration and management techniques, could be seen in the reduced requirement for seed purchase and drilling which can be a significant portion of restoration costs. The existing plant community does not appear to be degraded by early season nutrient reduction and thus will be a natural seed bank. Future research to explore nonfood sources of labile carbon could undoubtedly lower these costs significantly.

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Tables

Table 1: δ13C ratio and mass of sucrose treatments applied to one m^2 plots.

Treatment	Sucrose added (g m^{-2})	Carbon added (g m^{-2})	δ13C ratio
Control (Soil)	0	0	-26.96
Low C3	50	21	-24.97
Low C4	50	21	-11.95
Moderate C3	100	42	-24.97
Moderate C4	100	42	-11.95
High C3	250	105	-24.97
High C4	250	105	-11.95

Table 2: Tukey's HSD significance test of ammonium concentrations per week of treatment extracted from soils via 2M KCl and analyzed on segmented flow analyzer. Affect and duration of early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment and week as fixed factors and experimental block as a random factor

Ammonium			Tukey's HSD Comparison		
		n	Control	Low	Moderate
Week 3	Low	10	0.0752		
	Moderate	10	**0.0169**	0.9023	
	High	10	**0.0005**	0.2295	0.6259
Week 6	Low	10	0.2862		
	Moderate	10	0.1354	0.9643	
	High	10	**0.0147**	0.4259	0.7226
Week 9	Low	10	0.9836		
	Moderate	10	0.9851	1	
	High	10	0.9999	0.9816	0.9836
Week 15	Low	10	1		
	Moderate	10	0.9993	0.9996	
	High	10	0.9995	0.9979	0.992

Table 3: Tukey's HSD significance test of nitrate and nitrate concentrations per week of treatment extracted from soils via 2M KCl and analyzed on segmented flow analyzer. Affect and duration of early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment and week as fixed factors and experimental block as a random factor

Nitrate			Tukey's HSD Comparison		
		n	Control	Low	Moderate
Week 3	Low	10	0.3118		
	Moderate	10	0.4164	0.9975	
	High	10	**0.0211**	0.4732	0.3839
Week 9	Low	7	0.9522		
	Moderate	8	0.9998	0.9475	
	High	8	0.9179	0.4795	0.8109
Week 15	Low	9	0.9994		
	Moderate	9	0.9562	0.8668	
	High	9	0.9596	0.8744	1

Table 4: Primer Plus Permanova pairwise comparisons of Bray-Curtis similarity matrices using square root transformed plant community and soil microbial communities. Plant species in fewer than 10% of plots and PLFAs representing less than 4% of total removed.

Community Analyzed	Treatment	Control	Low	Moderate
Plant Community Composition	Low	**0.035**		
	Moderate	**0.0232**	0.2872	
	High	**0.0046**	0.1901	0.2909
Soil PLFA (Microbial) Composition	Low	0.3645		
	Moderate	0.3289	0.464	
	High	0.2805	0.7633	0.5009

Table 5: Tukey's HSD significance test of changes in V. dubia and other plant group cover determined in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment as fixed effects and experimental block as a random factor

Percent Cover Group/Species			Tukey's HSD Comparison		
		n	Control	Low	Moderate
Ventenata dubia	Low	10	0.3183		
	Moderate	10	**<.0001**	**0.0001**	
	High	10	**<.0001**	**<.0001**	0.1221
Native	Low	10	**0.0078**		
	Moderate	10	**0.009**	0.9999	
	High	10	**0.0117**	0.998	0.9994
Perennial	Low	10	0.8432		
	Moderate	10	0.7546	0.9969	
	High	10	0.5075	0.8965	0.9602
Other Annual	Low	10	0.7872		
	Moderate	10	0.8444	0.1505	
	High	10	**0.027**	0.0887	**0.0002**
Other Non-Native	Low	10	0.9828		
	Moderate	10	0.9996	0.9376	
	High	10	0.9701	0.7515	0.9753
Shannon Diversity	Low	10	0.9794		
	Moderate	10	0.5265	0.6346	
	High	10	0.3406	0.3952	0.9792
Richness	Low	10	0.9757		
	Moderate	10	0.9968	0.9942	
	High	10	0.9246	0.9942	0.9569

Table 6: Tukey's HSD significance test of changes in V. dubia and other common species seed production per inflorescence collecte in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment as fixed effects and experimental block as a random factor

Seeds per Inflorescence Species			Tukey's HSD Comparison		
		n	Control	Low	Moderate
V. dubia	Low	10	0.4622		
	Moderate	10	0.4899	0.9999	
	High	10	0.6192	0.9894	0.9942
A. americanus	Low	9	**0.032**		
	Moderate	9	**0.0012**	0.5062	
	High	9	**0.003**	0.7280	0.9851
P. secunda	Low	7	0.5468		
	Moderate	8	0.785	0.9605	
	High	8	0.9318	0.8545	0.9855
B. japonicus	Low	9	1		
	Moderate	9	0.9722	0.9492	
	High	9	0.9997	0.9996	0.9041
P. bulbosa	Low	10	1		
	Moderate	9	0.9951	0.9862	
	High	9	0.9868	0.9821	0.8951

Table 7: Tukey's HSD significance test of changes in V. dubia and other common species seed mass collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment as fixed effects and experimental block as a random factor

Seed Mass Species			Tukey's HSD Comparison		
		n	Control	Low	Moderate
V. dubia	Low	10	**<.0001**		
	Moderate	10	**<.0001**	**0.0309**	
	High	10	**<.0001**	**<.0001**	**0.0015**
A. americanus	Low	9	0.6608		
	Moderate	9	0.8458	0.9757	
	High	9	0.1725	0.7095	0.4355
P. secunda	Low	7	0.144		
	Moderate	8	0.9748	0.1601	
	High	8	0.5474	0.7747	0.6881
B. japonicus	Low	9	0.879		
	Moderate	9	0.6988	0.9732	
	High	9	0.8648	1	0.9816
P. bulbosa	Low	10	0.8287		
	Moderate	9	0.9687	0.3768	
	High	9	0.9622	0.9701	0.6618

Table 8: Tukey's HSD significance test of PLFA and NLFA lipid abundance of soils collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment as fixed effects and experimental block as a random factor

Lipid	Tukey's HSD	Control	Low	Moderate		Lipid	Tukey's HSD	Control	Low	Moderate
Total PLFA	Low	**0.0447**				Non-AMF fungi PLFA	Low	0.458		
	Moderate	0.1475	0.9136				Moderate	0.1052	0.7303	
	High	0.299	0.6716	0.9638			High	0.9795	0.1129	**0.0083**
Total NLFA	Low	1				Non-AMF fungi NLFA	Low	0.8219		
	Moderate	0.9407	0.8881				Moderate	0.9706	0.947	
	High	0.3987	0.1875	0.5582			High	0.5064	0.9021	0.6089
NLFA/PLFA	Low	0.9988				Non-AMF NLFA/PLFA	Low	0.9223		
	Moderate	0.8824	0.6736				Moderate	0.2075	0.2507	
	High	**0.0115**	**0.0004**	**0.0135**			High	0.6786	0.9038	0.6338
Gram positive	Low	**0.0458**				AMF PLFA	Low	0.1269		
	Moderate	0.1885	0.8555				Moderate	0.2823	0.9527	
	High	0.3419	0.6144	0.9736			High	0.5099	0.7328	0.9576
Gram negative	Low	**0.0223**				AMF NLFA	Low	1		
	Moderate	**0.0457**	0.9859				Moderate	0.8124	0.651	
	High	0.2603	0.5279	0.7391			High	0.2012	0.0614	0.5067
Cyclo/Gram-	Low	0.9994				AMF NLFA/PLFA	Low	0.8518		
	Moderate	0.9933	0.9979				Moderate	0.9995	0.6577	
	High	0.8513	0.6707	0.5578			High	0.2091	**0.0047**	0.0961
Actinomycetes	Low	0.4396				Bacteria PLFA/Fungi PLFA	Low	0.8185		
	Moderate	0.1387	0.838				Moderate	1	0.7264	
	High	0.867	0.8039	0.3149			High	0.1808	**0.0038**	0.0637

Table 9: Compound specific δ ^{13}C isotope analysis of PLFA and NLFA extracted from high C3 and C4 sucrose derived treatment plots. P-value <0.05 indicates a significant difference in the isotope signature between the treatments. This indicates if the microbial group readily assimilated labile carbon treatments. PLFAs are indicators of microbial cell wall abundance (biomass) while NLFAs indicate the abundance of fungal storage lipids. Isotope data are reported in delta notation compared to Vienna Pee Dee Belemnite.

Microbial Group			p-value
	Sucrose Type	n	C4
Gram negative bacteria PLFA	C3	4	**0.0342**
Gram positive bacteria PLFA	C3	4	**0.0499**
Actinomycetes bacteria PLFA	C3	4	**0.0448**
Fungi PLFA	C3	4	**0.0151**
AMF PLFA	C3	4	0.1012
Other eukaryotes PLFA	C3	4	**0.0009**
Fungi NLFA	C3	4	0.1216
AMF NLFA	C3	4	0.6766
Other eukaryotes NLFA	C3	4	0.2157

Table 10: Tukey's HSD significance test of soil moisture at week twelve post application and plant parasitic nematode abundance at week 12 and 15 as an effect of early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m^2 experimental plots. Results from mixed models with treatment and week as fixed factors and experimental block as a random factor.

Soil Moisture and Plant Parasitic Nematodes			Tukey's HSD Comparison		
		n	Control	Low	Moderate
Soil Moisture Week 12	Low	10	0.525		
	Moderate	10	0.4385	0.9982	
	High	10	**0.0374**	0.3632	0.4636
Plant Parasites Week 12	Low	10	**0.0357**		
	Moderate	10	**0.0122**	0.9702	
	High	10	0.0762	0.9841	0.8516
Plant Parasites Week 5	Low	10	0.8109		
	Moderate	10	**0.0299**	0.0985	
	High	10	0.0823	0.2607	0.9643

Table 11: Anova table of soil respiration as an effect of early spring labile carbon treatments as sucrose (control 0 g, low 50 g, moderate 100 g, and high 250 g) applied to 1 m2 experimental plots. Respiration analyzed weekly throughout the growing season using Licor IRGA. Results from mixed models with treatment and week as fixed effects and experimental block as a random effect.

	numDF	denDF	F-value	p-value
(Intercept)	1	480	97.97285	<.0001
Week	13	480	45.99823	<.0001
Treatment	3	28	0.37423	0.7722
Week:Treatment	39	480	1.02989	0.4238

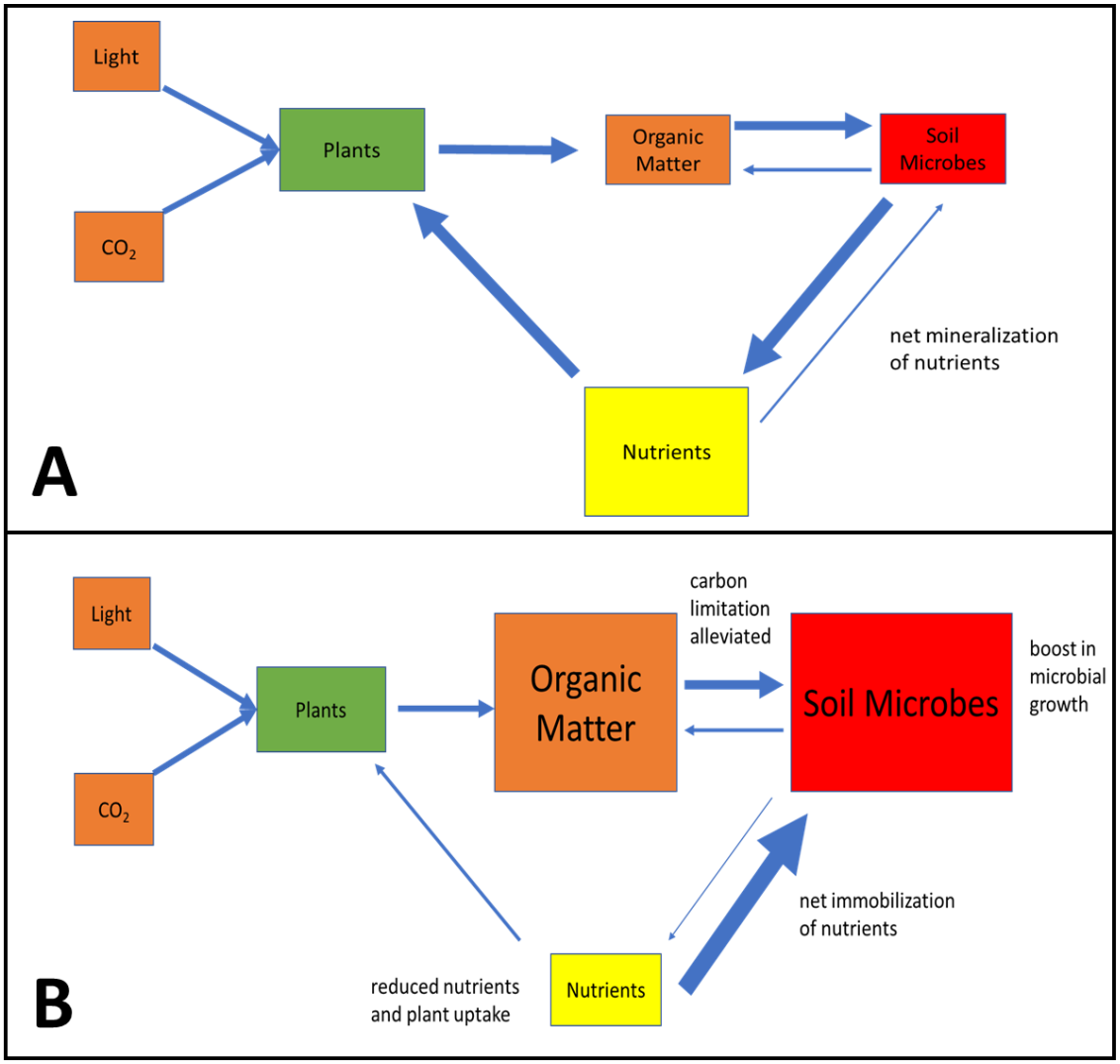

Figure 1: Top pane (A) shows energy and nutrient flow in a system where soil microbes are energy limited. Lower pane (B) shows how applying carbon (organic matter) can increase soil microbial abundance and cause reduced nutrient availability to plants via increased competition with soil microbial community.

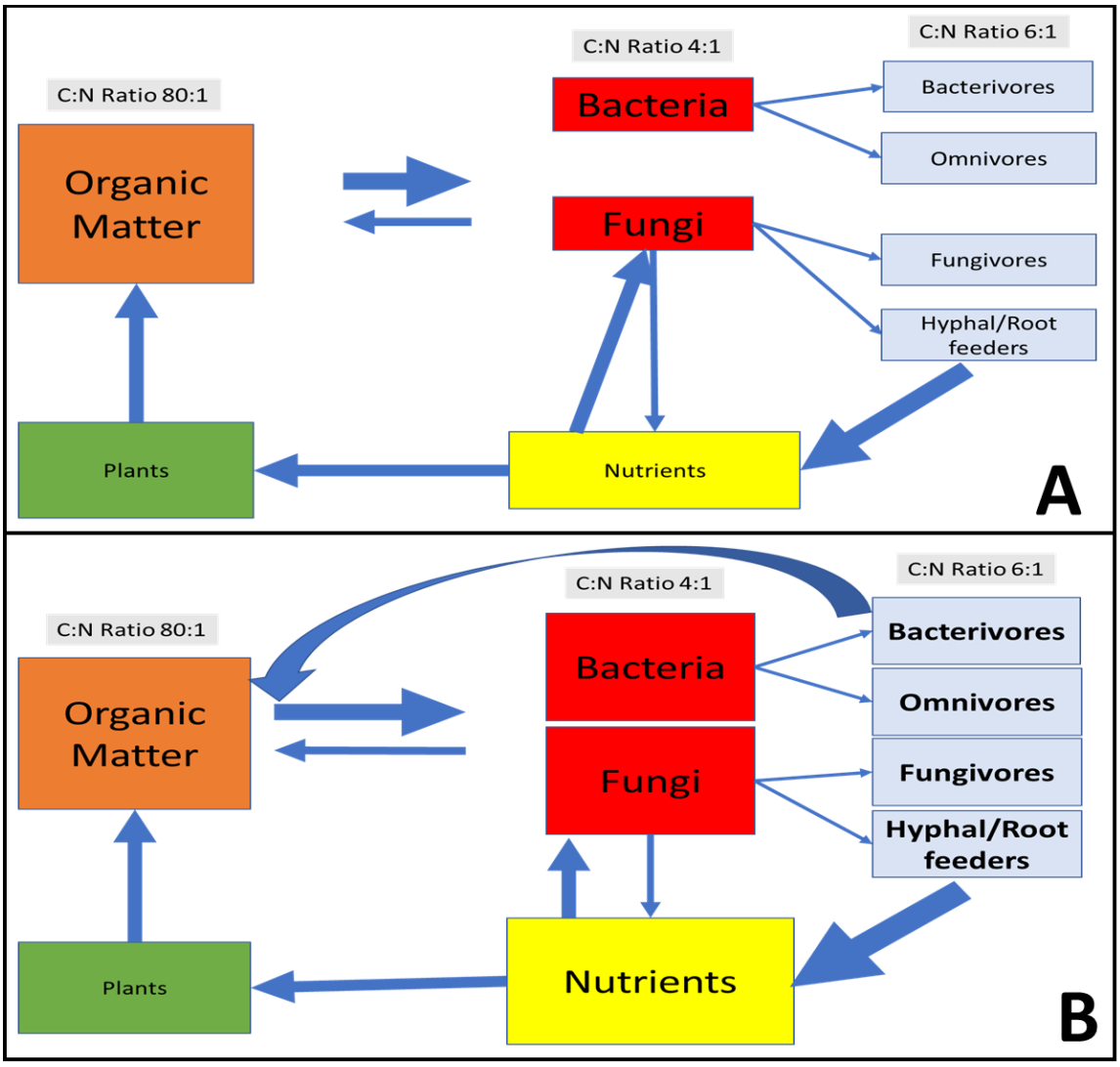

Figure 2:Top pane (A) shows energy and nutrient flow in a system where soil microbes are energy limited and includes the impact of higher trophic levels on nutrient availability. Lower pane (B) shows how applying carbon (organic matter) can increase soil microbial abundance and cause reduced nutrient availability to plants via increase but with increased predator abundance nutrient availability will likely increase as well resulting in less of a nutrient reduction effect or shorter duration.

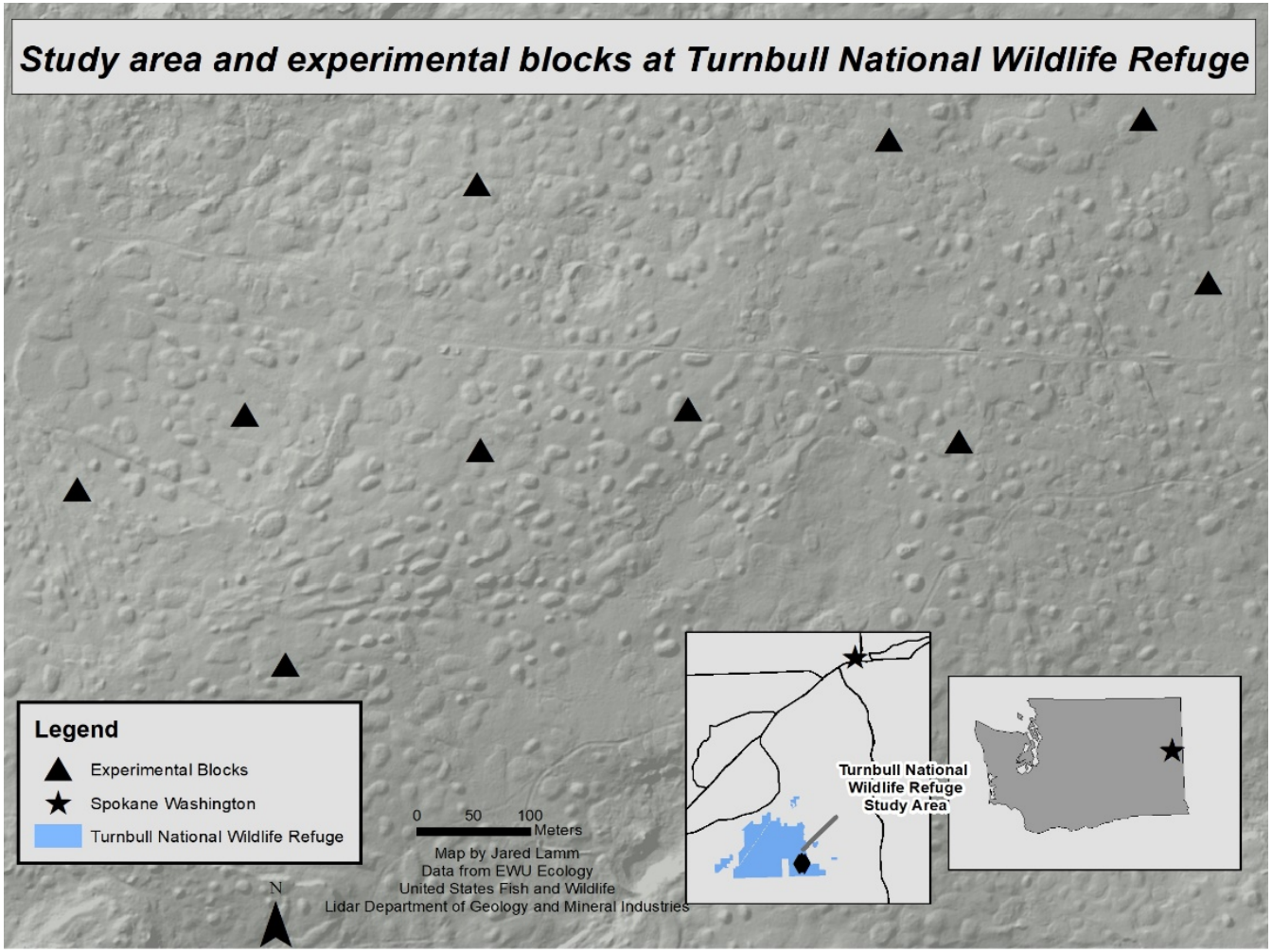

Figure 3: Study area at Turnbull National Wildlife Refuge in Eastern Washington. Experimental replicates (blocks) indicated by black triangles. Each block consists of seven 1 m^2 plots separated by 0.5 m. Experimental area is in a Mima mound dominated region of semi-arid grasslands. Experimental units were placed in between mounds on soils a minimum of 5 cm depth and were separated by at least 100 m.

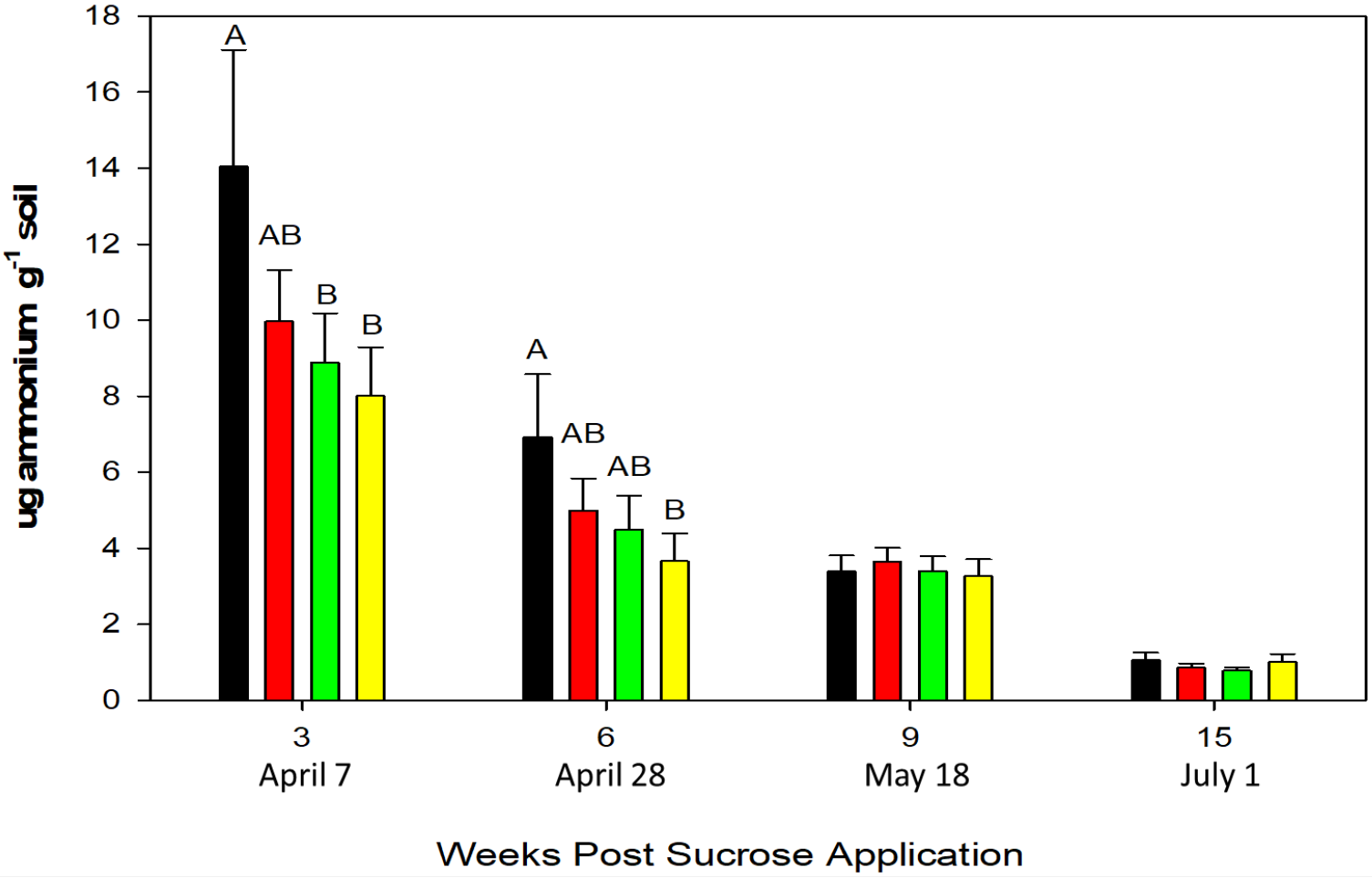

Figure 4: Ammonium concentrations per week of treatment extracted fropm soils via 2M KCl and analyzed on segmented flow analyzer. Means are estimated marginal means of mixed models with treatment and week as fixed factors and experimental block as a random factor. Error bars are +/- one SE from mixed model. Star indicates weeks with significant difference between control and labile carbon treatments.

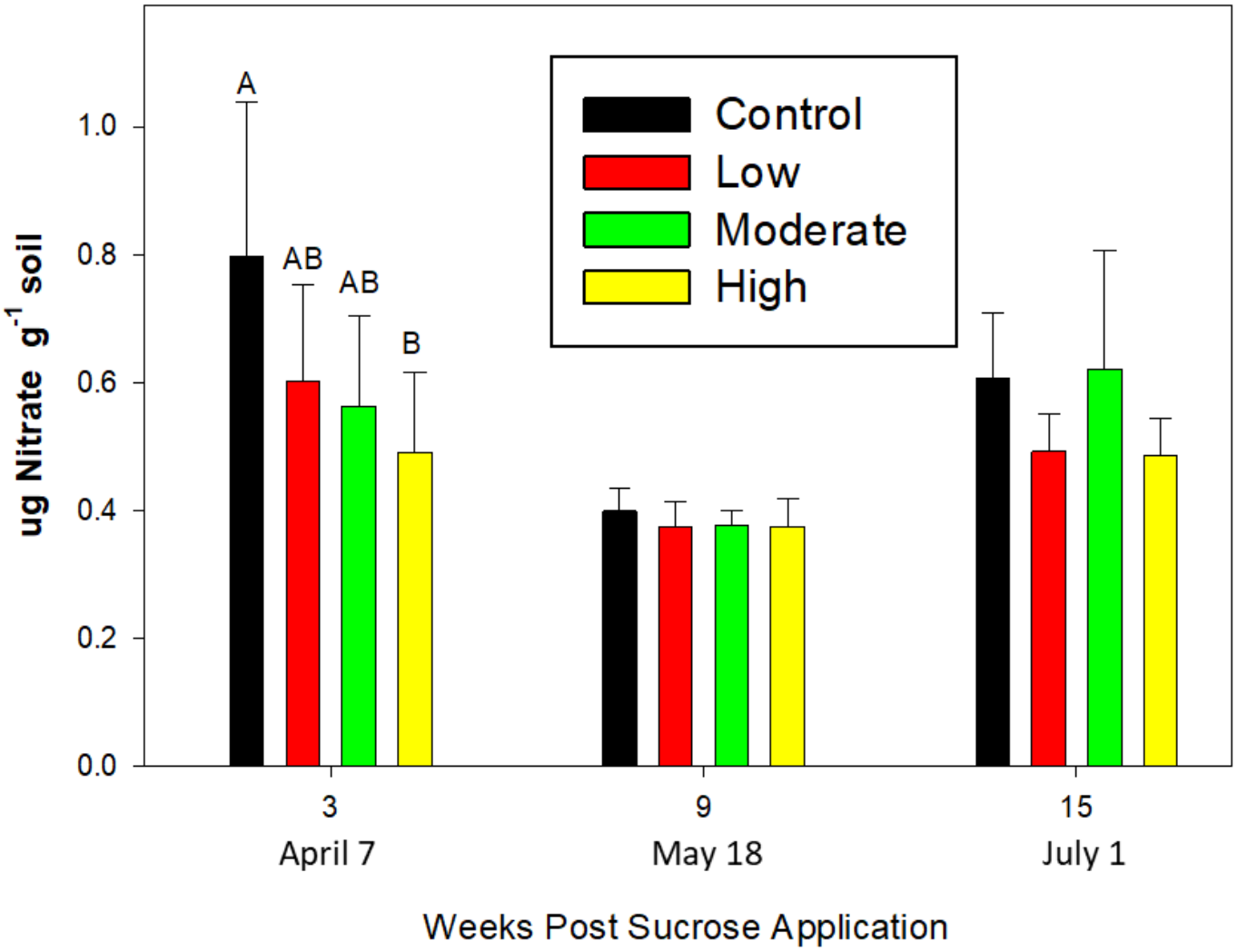

Figure 5: Nitrate and nitrte concentrations of treatment extracted fropm soils via 2M KCl and analyzed on segmented flow analyzer. Means are estimated marginal means of mixed models with treatment and week as fixed factors and experimental block as a random factor. Error bars are +/- one SE from mixed model. Star indicates weeks with significant difference between control and labile carbon treatments.

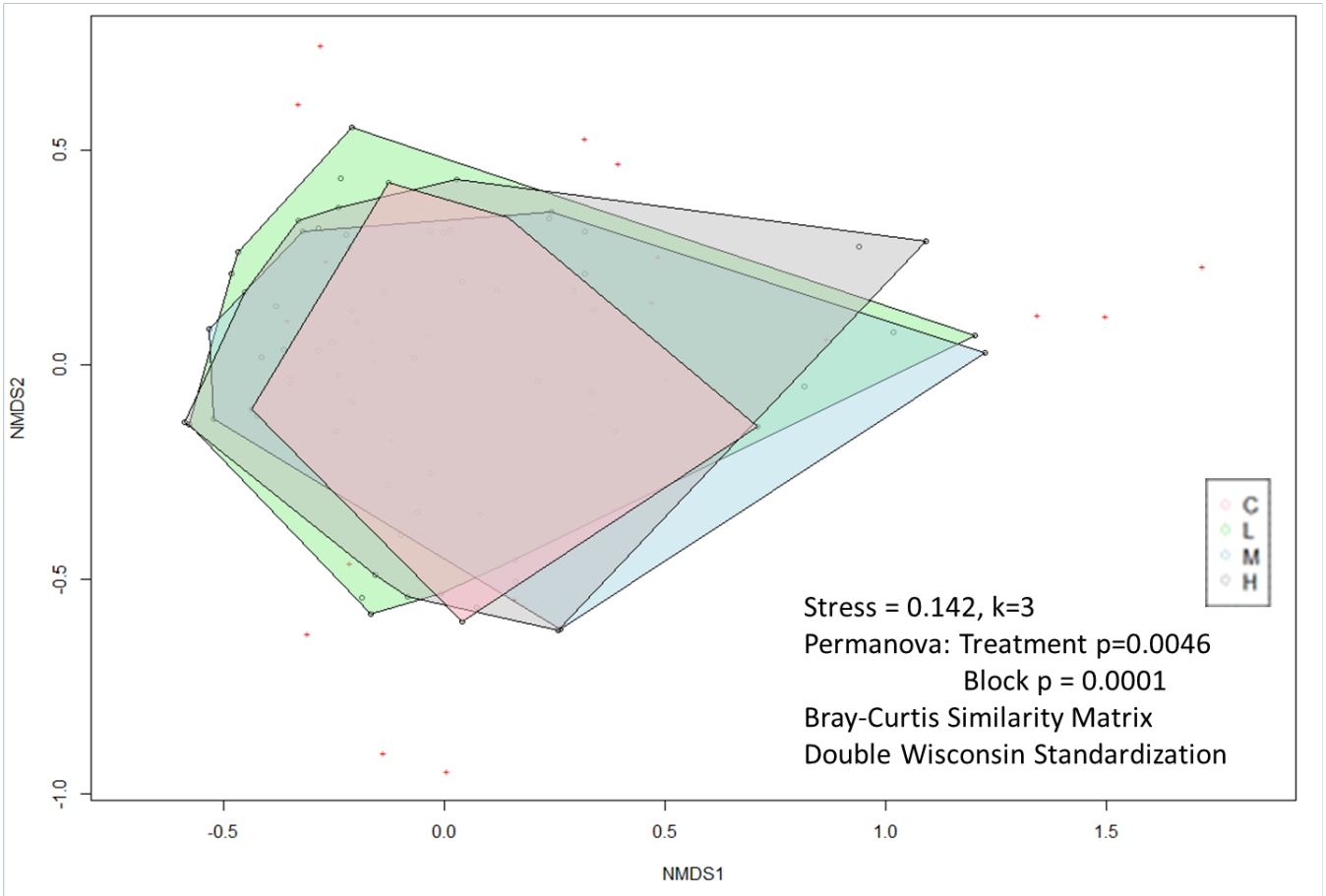

Figure 6: NMDS of plant community composition. Species values were Wisconsin double standardization transformed before calculating a resemblance matrix using Bray-Curtis similarities after removing species present in fewer than 4 plots. NMDS performed in R version 3.5.1 using vegan package (R Core Team 2019). Stress = 0.142 k=3. Two solution reached after 20 runs. Non-metric R^2 = 0.98, Linear fit R^2 = 0.871. Treatments were significantly different from control (Permanova, p= 0.0046) using square root transformed plant abundance data, all other calculations were held the same as the NMDS. Shaded polygons are the labile carbon treatments. Smaller polygons are experimental blocks n=10.

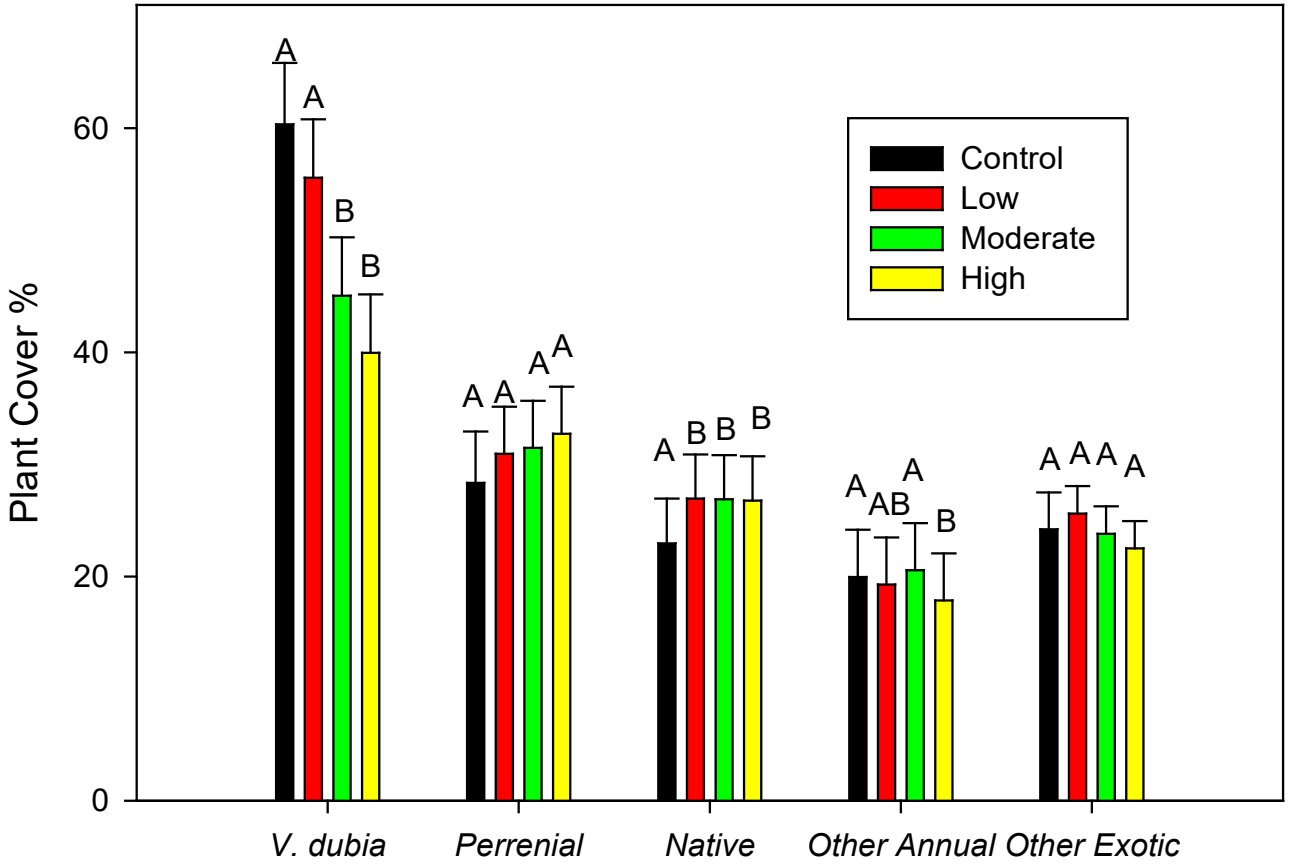

Figure 7: Percent cover of Ventenata dubia and other plant groups in 1 m^2 experimental plots determined in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within species or plant group.

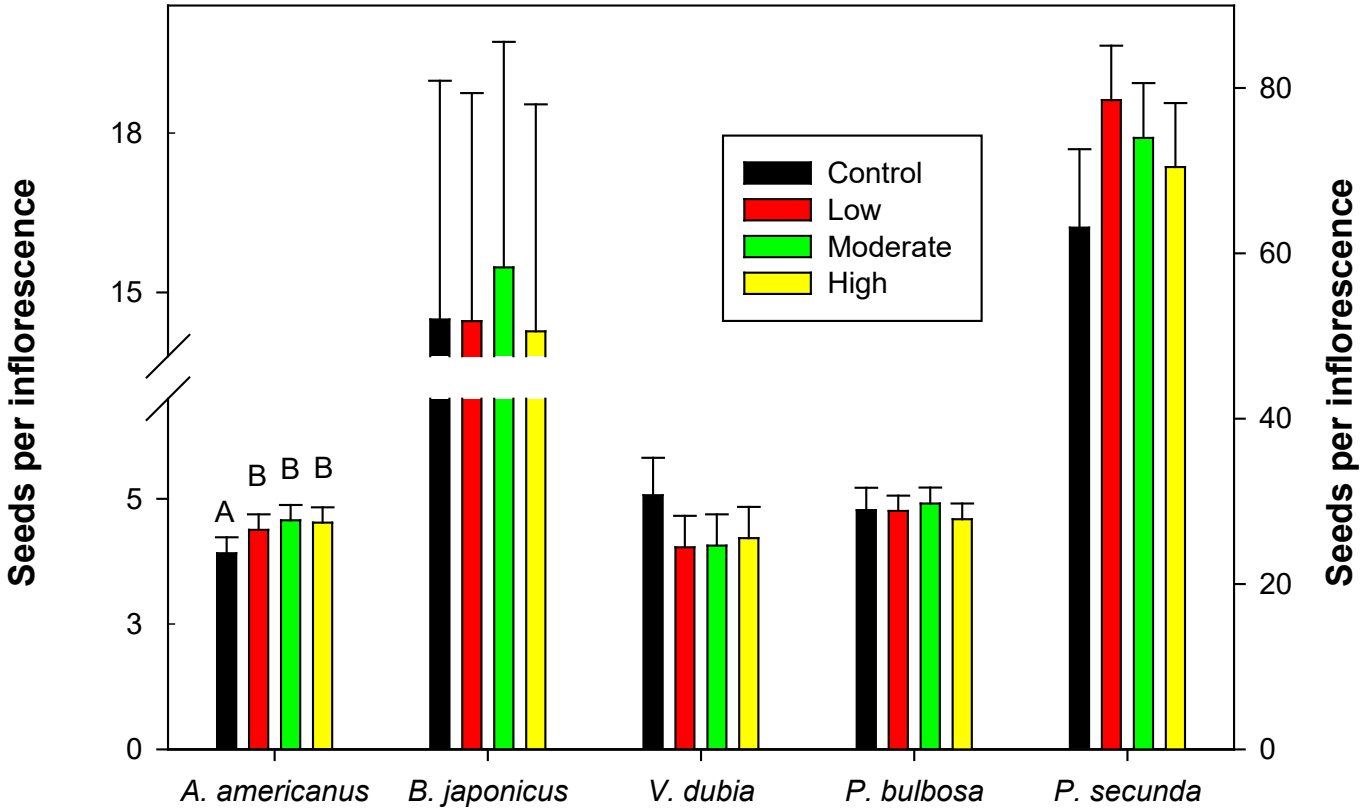

Figure 8: Average seeds per inflorescence of the two largest inforesceneces per quadrant (total of eight) of each 1 m^2 experimental plot collected from the five most ubiquitous species in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within species.

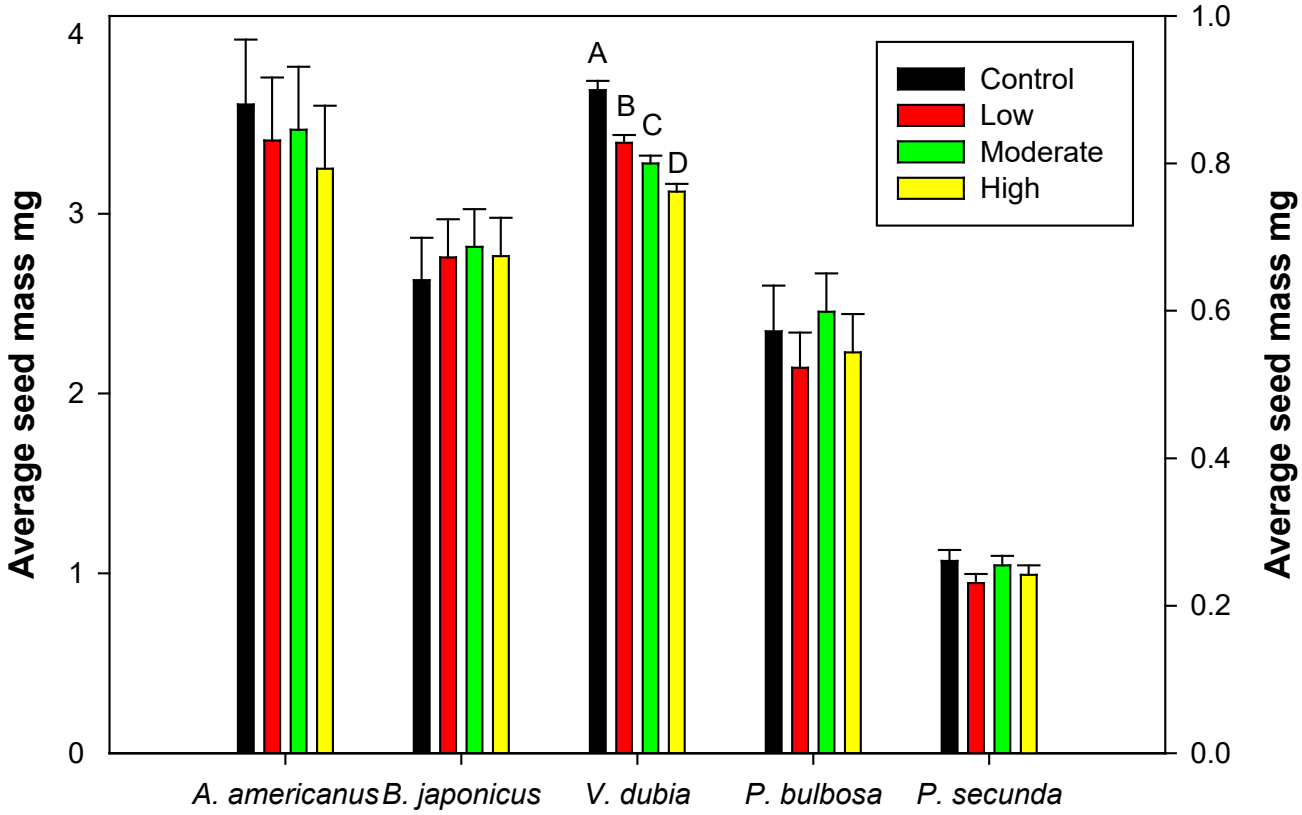

Figure 9: Average seeds mass of the two largest inforesceneces per quadrant (total of eight) of each 1 m^2 experimental plot collected from the five most ubiquitous species in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within species.

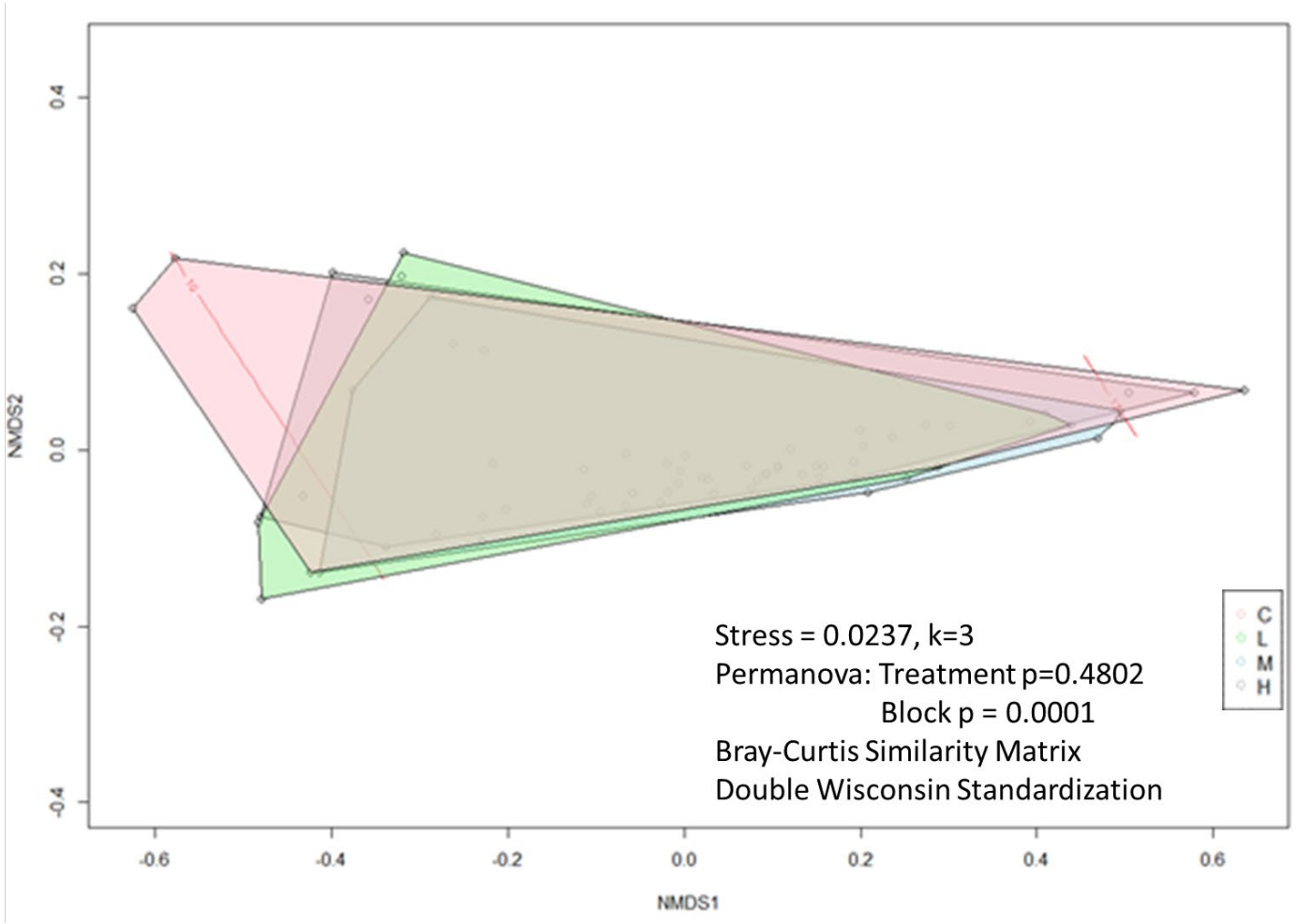

Figure 10: NMDS of soil lipid profile. Lipid values were Wisconsin double standardization transformed before calculating a resemblance matrix using Bray-Curtis similarities. Isohyets in background show soil moisture at 9 weeks after treatment applications throughout the surface. NMDS performed in R version 3.5.1 using vegan package (R Core Team 2019) Stress = 0.0238 k=3. Two convergent solutions reached after 20 runs. Non-metric R^2 = 0.999, Linear fit R^2 = 0.998 Treatments were not significantly different from control (Permanova, p= 0.4802) using square root transformed lipid abundance data, all other calculations were held the same as the NMDS.

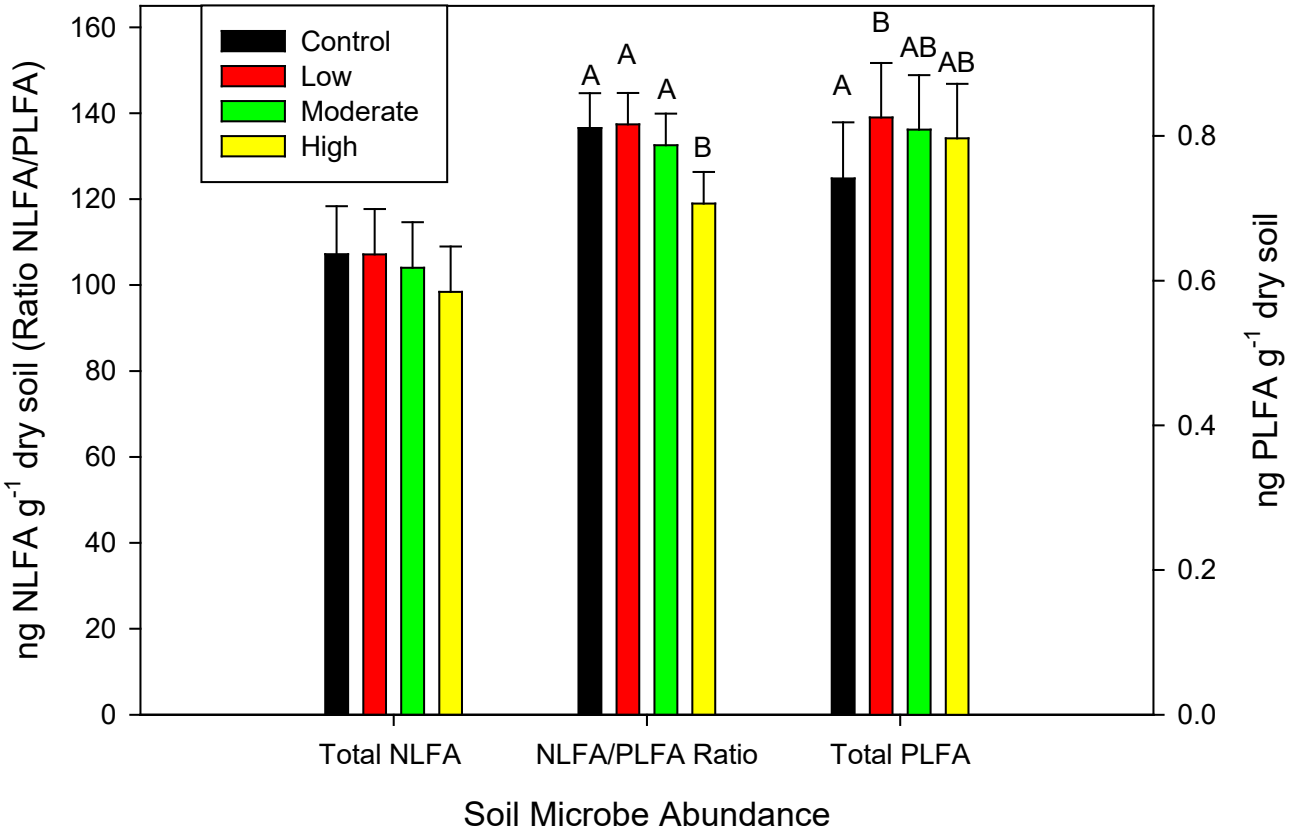

Figure 11: Abundance of lipids in soils from 1 m^2 experimental plot collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within each group.

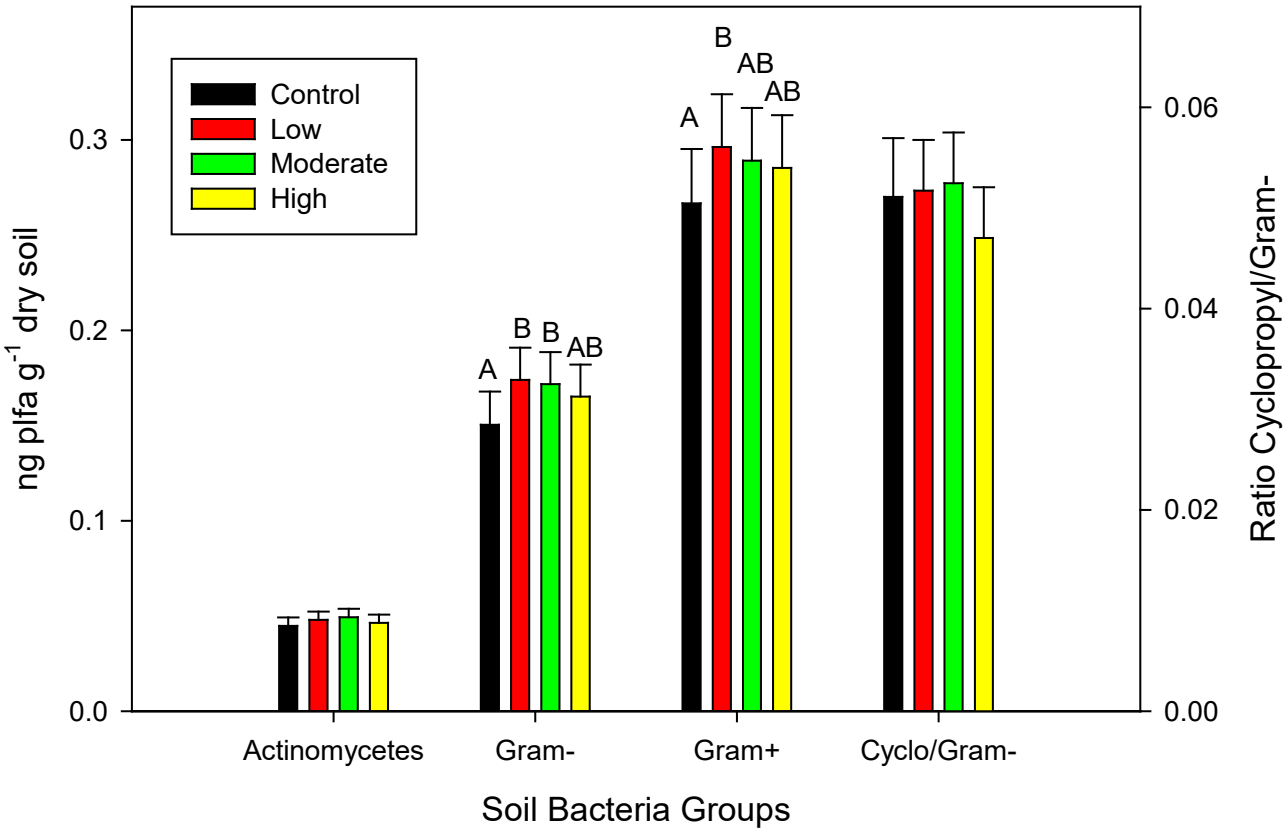

Figure 12: Abundance of lipids in soils from 1 m^2 experimental plot collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within each group.

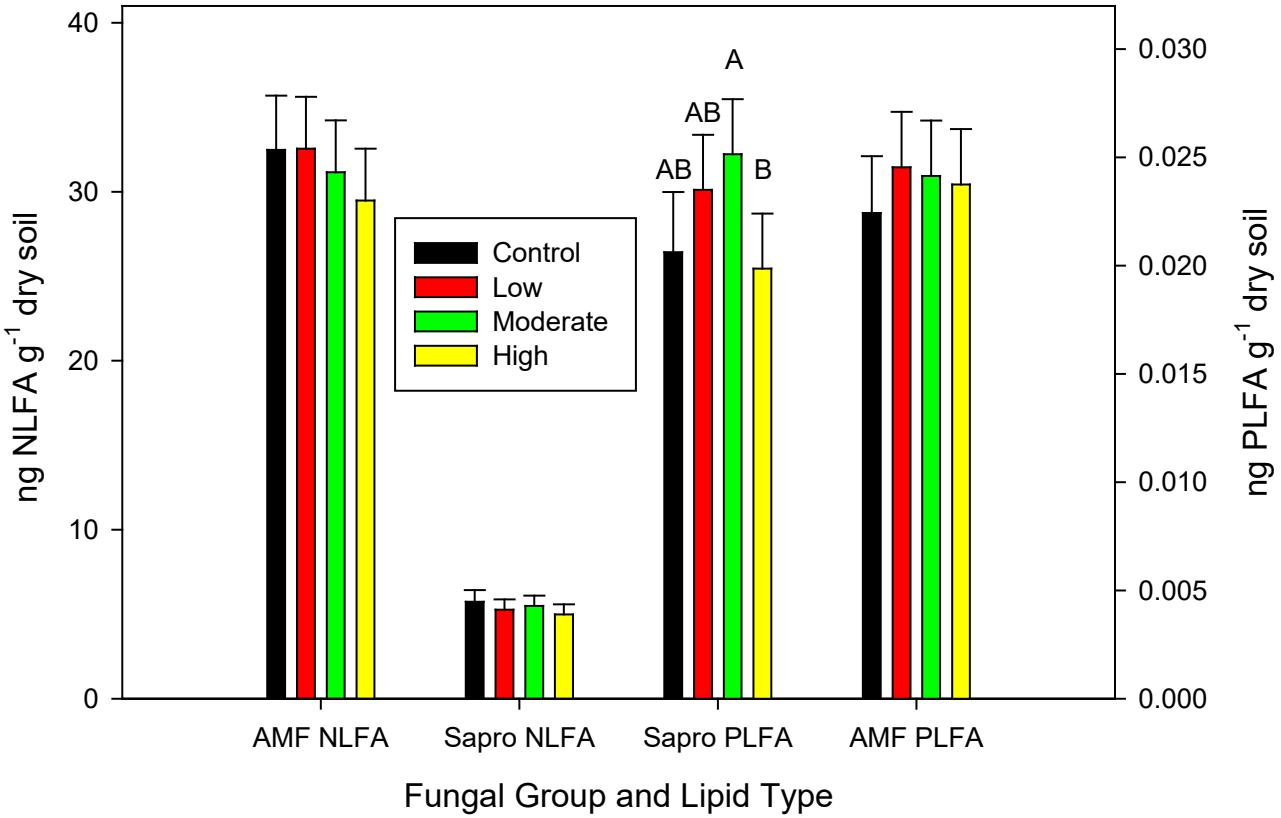

Figure 13: Abundance of lipids in soils from 1 m^2 experimental plot collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within each group.

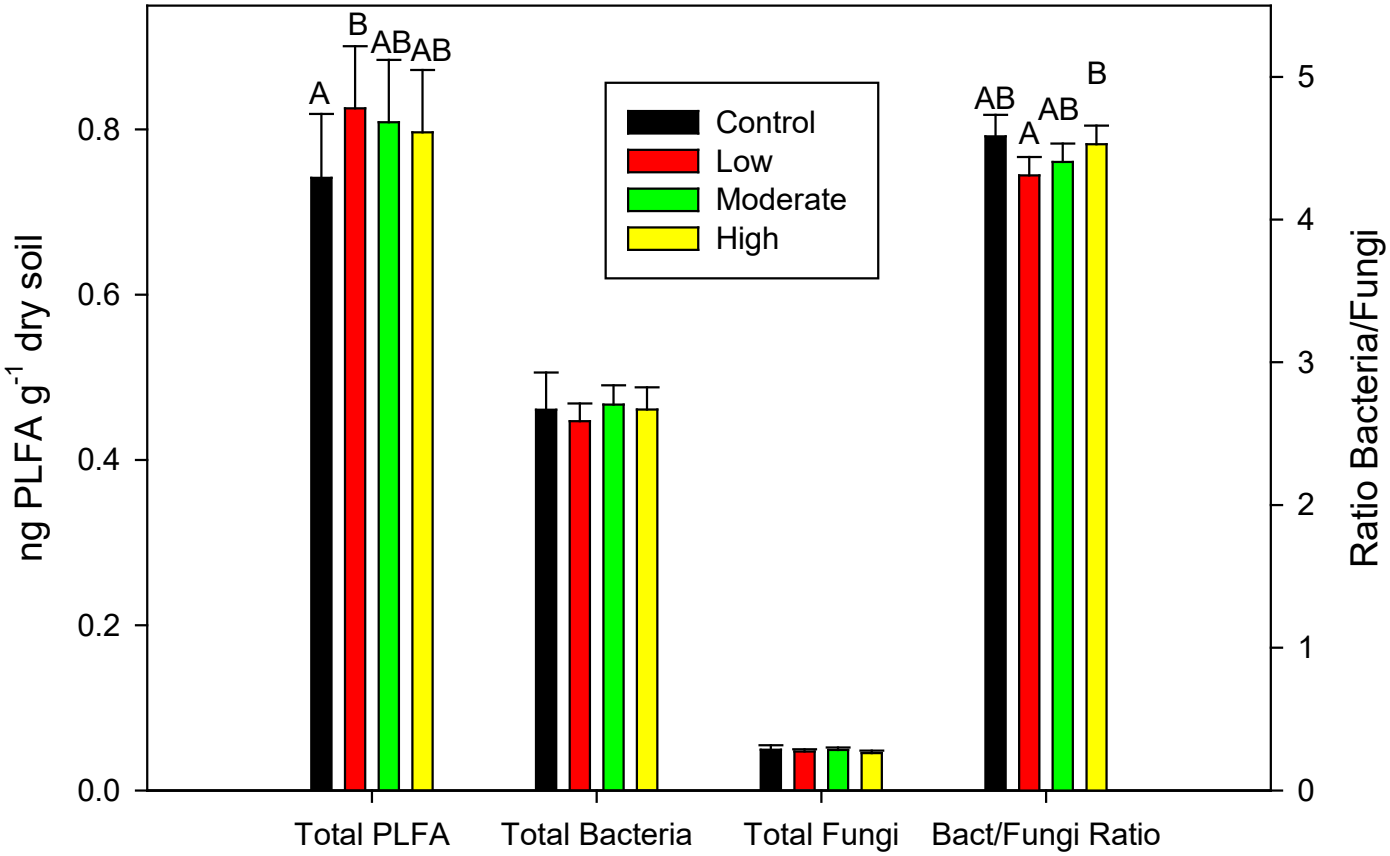

Figure 14: Abundance of lipids in soils from 1 m^2 experimental plot collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within each group.

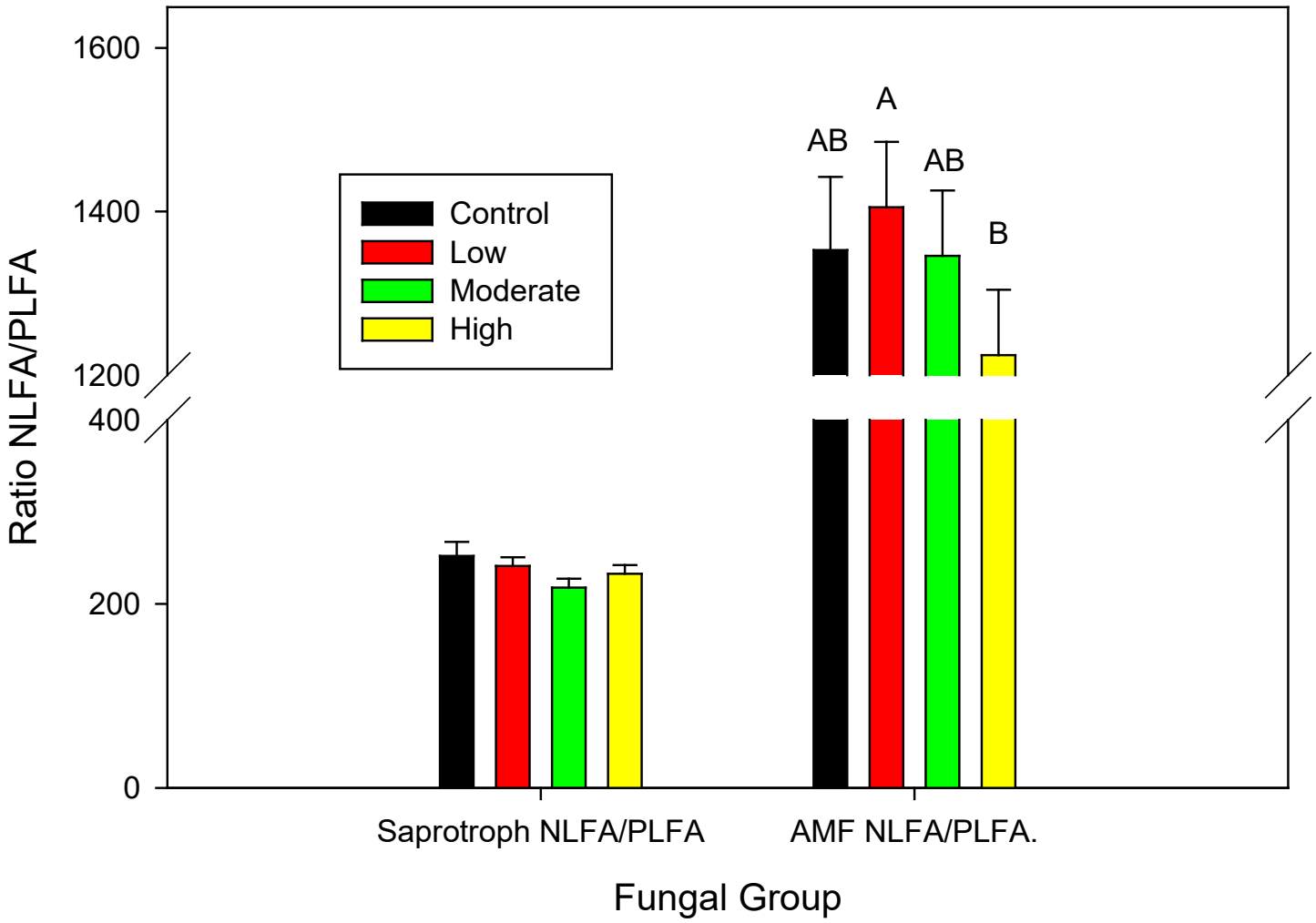

Figure 11: Abundance of lipids in soils from 1 m^2 experimental plot collected in July following early spring labile carbon treatments as sucrose (contol 0 g, low 50 g, moderate 100 g, and high 250 g). Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between control and labile carbon treatments within each group.

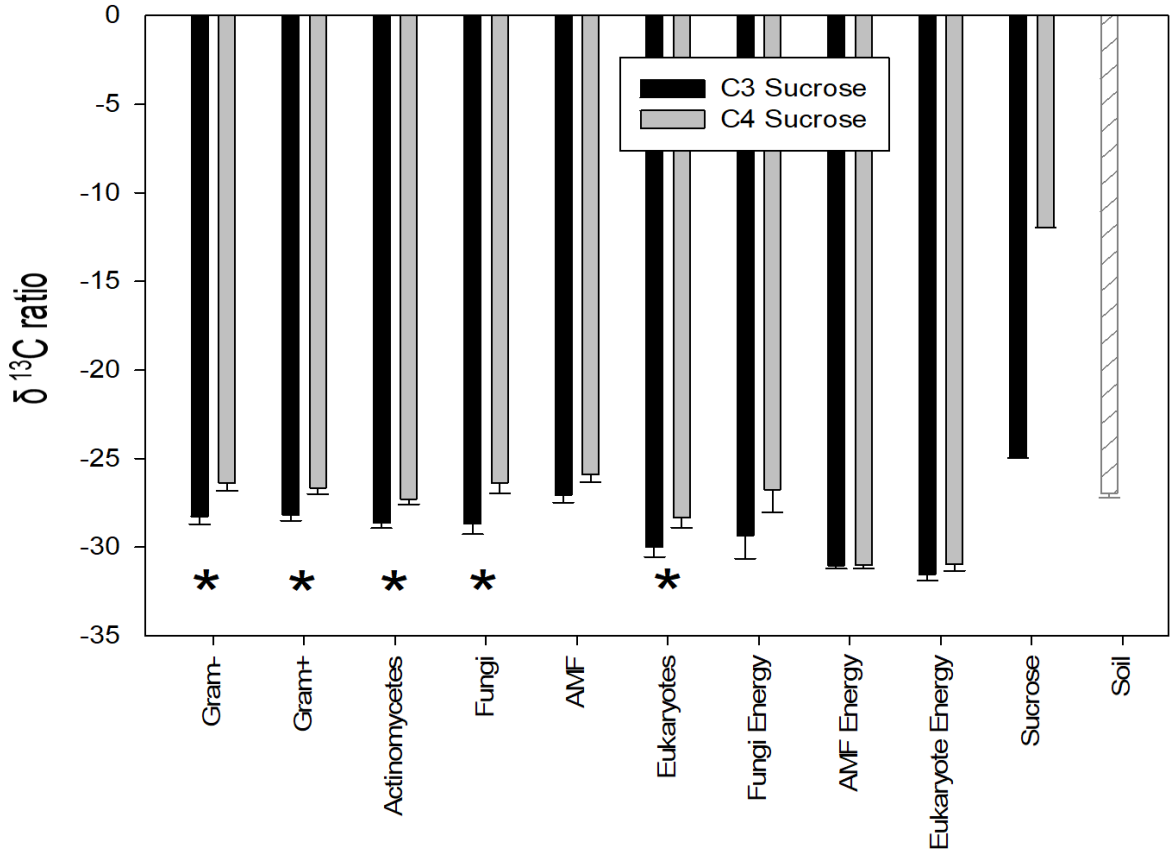

Figure16: δ 13C ratio Vienna Pee Dee Belemnite of PLFA and NLFA lipids for soil microbial groups in soils from 1 m2 experimental plots collected in July following early spring high (250g) labile carbon treatments as C3 or C4 derived sucrose Means are estimated marginal means of mixed models with treatment as the fixed effect and experimental block as a random factor. Error bars are +/- one SE from mixed model. Different letters indicate significant difference between sucrose type within each group. Also shown are the δ 13C ratios of the two types of sucrose applied and the average background soil δ 13C ratio.

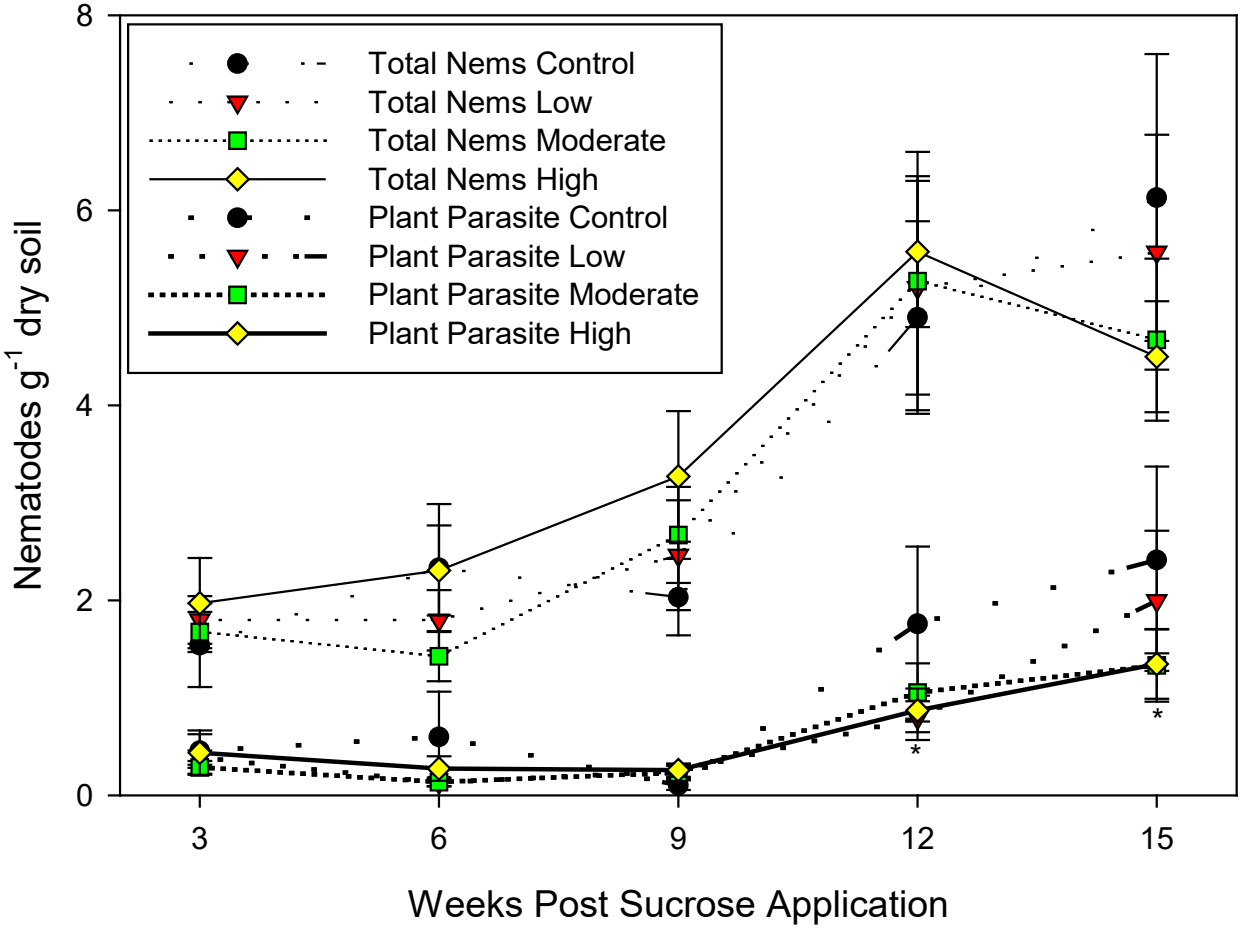

Figure17: Total nematode and plant parasitic nematode abundances determined every three weeks throught the growing season. Means are estimated marginal means of mixed models with treatment and week as fixed factors and experimental block as a random factor. Error bars are +/- one SE from mixed model. Star indicates weeks with significant difference between control and labile carbon treatments.

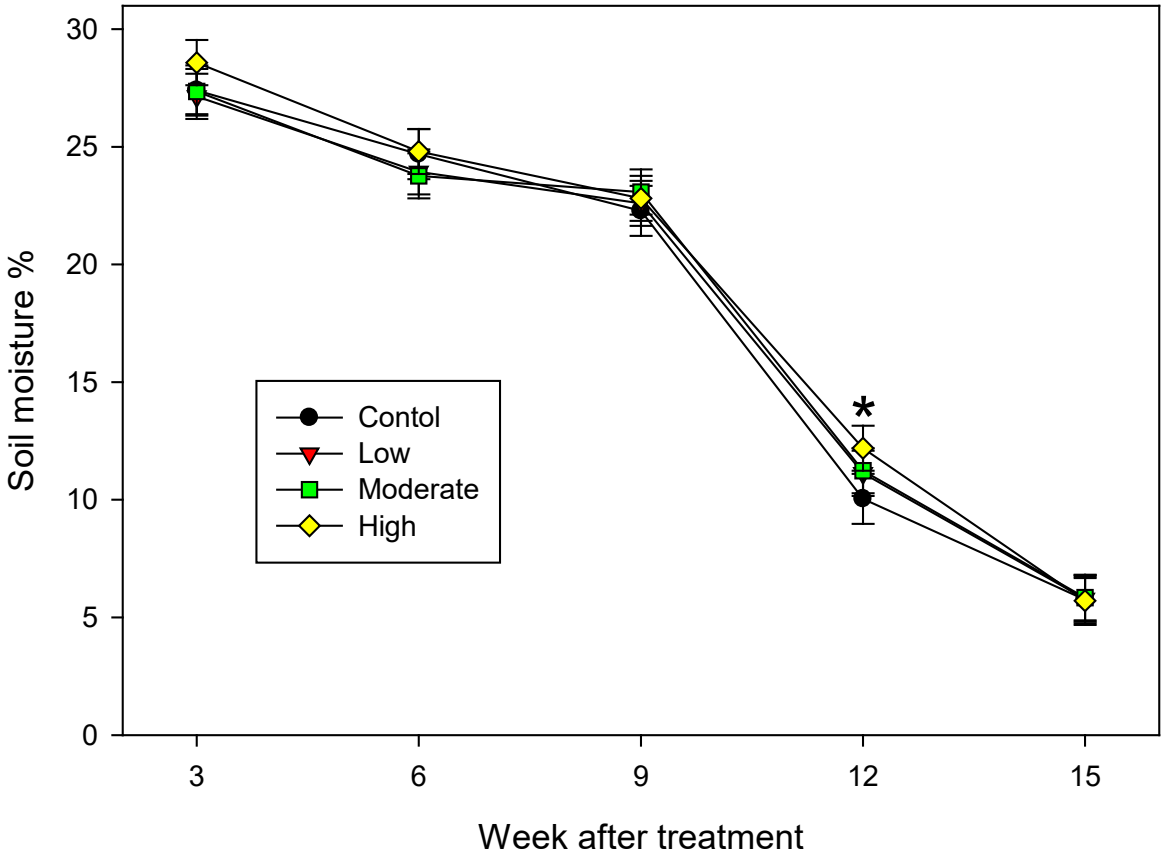

Figure18: Soil moisture determined gravimetricly every three weeks throught the growing season. Means are estimated marginal means of mixed models with treatment and week as fixed factors and experimental block as a random factor. Error bars are +/- one SE from mixed model. Star indicates weeks with significant difference between control and labile carbon treatments.

VITA

Jared F. Lamm

Education

Master of Science **Eastern Washington University** **2019**
Ecology/Soil Ecology

Bachelor of Science **Eastern Washington University** **2017**
Environment Science Biology *GPA: 3.97/4.00*
Minor Biology

Graphical Information Systems **Eastern Washington University** **2017**
Certificate

Research Experience and Skills

Targeted short-term nutrient reduction to manage Ventenata dubia *Plant/Soil Ecology*

- Plant and soil microbial community analysis
- Soil nutrient availability

Soil and Water Quality Analysis *Geochemistry Lab*

- Developed Standard Operating Procedures for sample intake and extraction procedures in a three-person lab
- Performed and analyzed plant and soil digests and leaches via ICP-OES
- Instrument calibration, quality control, check standards and serial dilutions

Teaching Experience

Graduate Fellow Teaching Assistant **Eastern Washington University**
Fall 2017–Spring2019

- Design, setup, and execute labs for environmental science, general biology, and experimental design classes.

Undergraduate Teaching Assistant **Eastern Washington University**
Fall 2016-Spring 2017

Introduction to Environmental Science Lab. Prepared lab materials and equipment, provided instruction on groundwater, greenhouse gases and water properties analysis, graded and submitted scores for labs.

Fellowships, Honors, and Memberships

Eastern Washington University (EWU) Biology Graduate Fellowship 2017
EWU Outstanding Environmental Science Graduate 2017
Turnbull Scholars Fellowship EWU 2016

Society for Ecological Restoration member	2018
Washington Native Plant Society member	2018
Northwest Scientific Association member	2018

Funding

Northwest Scientific Association	2018
Washington Native Plant Society Conservation Grant	2018
Society for Ecological Restoration	2018

Presentations

Lamm, J. F. Bastow, J. Brown, R. "Are plant soil feedbacks in semi-arid grasslands altered by the invasive winter annual grass, *Ventenata dubia*?" **Poster Presentation**. Ecological Society of America, August 2017

Lamm, J. F. Bastow, J. Brown, R. "Are plant soil feedbacks altered by the invasive grass, *Ventenata dubia*?" **Power Point Presentation**. Eastern Washington University Research and Creative Works Symposium, May 2017

Lamm, J. F. Nezat, C. "Biologically Available Elements of Soils Invaded by *Ventenata dubia* at Turnbull National Wildlife Refuge, Washington." **Poster presentation**. NCUR, April 2017

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