Proposed Titles:

ASSESSING RATES OF DECOMPOSITION AND NITROGEN LOSS IN AN ECONOMICALLY VIABLE COVER CROP

BIOMASS AND NITROGEN LOSS OF VARIOUS COVER CROPS IN CENTRAL ILLINOIS

SURFACE DECOMPOSITION AND NITROGEN LOSS OF TWO COMMON AND TWO NOVEL COVER CROPS IN CENTRAL ILLINOIS

DEVELOPING AN UNDERSTANDING OF THE ECOSYSTEM EFFECTS OF GENETICALLY MODIFIED PENNYCRESS THROUGH DECOMPOSITION AND NITROGEN LOSS

ABSTRACT

Current farming practices, particularly American Midwest, are reliant on nutrient application to increase production. This practice is unsustainable. When paired with other unsustainable practices the agricultural land of the midwest is subject to substantial nutrient and soil losses. Cover cropping is a sustainable farming practice used to reduce nutrient, water, and soil losses from agricultural land. Through the use of cover crops, the total nitrogen (N) load leaving agricultural systems can be greatly reduced at the benefit of downstream marine ecosystems. However, cover cropping is not an economically viable practice and provides little incentive for widespread implementation. Pennycress (*Thlaspi arvense* L.) is an oilseed producing winter cash crop that has the potential to serve as a nutrient mitigating cover crop. To better understand the ecosystem effects of pennycress, we analyzed the rate of decomposition and N loss in pennycress, genetically modified pennycress (*AOP2*  pennycress), cereal rye, and annual rye. We conducted an experimental study at the Illinois State University Farm blocked by soil type with 9 replicates used to account for pseudoreplication. Biomass was collected from cereal rye, annual rye, and wild type pennycress from the Illinois State University Farm in Lexington, Illinois. *AOP2* pennycress biomass was collected at Western Illinois University. Biomass was roughly chopped and placed into 396 mesh forage bags. The bags were placed on the soil surface of two distinct soil types representative of Central Illinois with a small amount of soil placed on top of the bag. Results demonstrated XXXXXXX

INTRODUCTION

The quality and productivity of many coastal marine ecosystems in the Gulf of Mexico is heavily influenced by riverine water flowing into these systems. Nutrient loads from agricultural sources contribute more than 70% of Nitrogen (N) and Phosphorus (N) delivery to the gulf with corn and soybean production comprising 52% of this N load (Alexander et al. 2007). While some of this N is a product of suburban landscapes, water reclamation districts, and industrial activities much is demonstrably attributed to agricultural practices. Much of the N applied to agricultural land is lost to the environment. Cash crops uptake roughly half of applied inorganic fertilizer and estimates show that in the United States up to 34% of the nitrogen fertilizer applied to crops is lost to the environment costing an estimated $400 million in annual N loss (Boehm 2020, Robertson & Vitousek 2009). Total N load to the gulf originates primarily from Minnesota, Illinois, Iowa, Indiana, and Ohio (Donald et al. 2001). N percolates through the soil profile and into subsurface drainage systems where it moves from drainage ditches to streams and major river systems where it is deposited into coastal marine ecosystems. Here it stimulates algal production creating algal blooms that are aerobically decomposed forming hypoxic zones. The sensitivity of gulf coast ecosystems to N pollution is increasing. Estimates demonstrate that up to a 70% reduction of Spring total nitrogen loads from the 1988-1996 period may be required to meet reduction goals (Liu et al. 2010). In addition to nutrient losses from agricultural systems, soil losses are considerable and poorly understood. Research suggests that soil losses have been significantly underestimated and between 24% and 46% of the corn belt has completely lost A-horizon soil (Thaler et al 2020).

While marine ecosystems are nutrient limited by N, freshwater ecosystems are nutrient limited by phosphorus (Hans 2009) . This often makes the local effects of N pollution negligible while downstream effects are considerable. The necessity for widespread implementation of sustainable farming practices is undeniable. Myriad challenges constrain implementation of sustainable policy and practices. Farms are primarily concerned with income and production. Because of this, bottom up practices are contingent on economic incentive to receive widespread implementation. Additionally, as local effects of nutrient pollution remain less severe than downstream effects, top down legislation at the source of pollution receives little support. It is unreasonable to expect farms to enact sustainable practices of their own accord or for legislators to enact legislation supporting sustainable farming practices. The unfortunate reality of protecting the integrity of the Earth to support human life is that to do so requires the parties responsible for environmentally damaging practices to receive economic benefit from sustainable and environmentally sound practices.

~~In addition to agriculture, water reclamation districts and industrial activities can play a considerable part in freshwater pollution. For instance, although wastewater treatment plants remove between 94-98% of E.coli from water, E.coli has been identified in all samples of treated wastewater (Brechet et al. 2014). Additionally, sewage sludge from industrial activities often contains heavy metals that bioaccumulate and can pose significant environmental and human health concerns (Malwina 2019). However, the purpose of this research is sustainable agriculture which will do little to abate pollution from water reclamation districts and industrial practices.~~

There are three sustainable agricultural practices that show the most promise in reducing nutrient and soil loss: buffer strips, the 4 R’s of nutrient management, and cover cropping. Buffer strips refer most generally to vegetative barriers that slow runoff, reduce erosion, and remove contaminants from water and the soil (Dabney et al. 2006). While buffer strips can include in-field and after-field buffer strips, we focus on edge-of-field buffer strips as they are colloquially most common and better suited to the geography of Illinois. Edge-of-field buffer strips include field borders, filter strips, and vegetative barriers. These are strips of vegetation designed to mitigate nutrient and erosion loss (Debney et al. 2006). The 4 R’s of nutrient management refer to the right source, right rate, right time, and right place. They combine economic, environmental, and social facets of nutrient management. Understanding and implementing these can help reduce nutrient pollution by keeping nutrients on the field (Rogers 2019). Cover crops replace the annual fall fallow period and are beneficial for soil retention and nutrient mitigation (Poeplau & Don 2015). By replacing fallow periods cover crops have positive impacts on nutrient mitigation and soil quality. They improve soil quality by providing food for soil microorganisms, reducing sediment production, and increase the cation exchange capacity, aggregate soil stability, and water infiltrability of soils (Dabney et al 2001). Cover crops uptake excess nutrients that could leach into subsurface drainage systems or leave a system through surface runoff. This makes them effective tools for nutrient mitigation and soil management. Each of these sustainable farming practices has the potential to reduce nutrient pollution and improve soil quality, but none have seen wide implementation in the midwest.

Cover crops have the most potential to receive widespread implementation. They are highly effective at mitigating nutrient and soil loss and can do so over a wide array of geographic and climate conditions. However, cover crops are poorly utilized. The reasons for this are multifaceted. One of the primary drivers is economics. Few sustainable agriculture practices are of economic value to a farmer. Moreover, 54% of the cropland in the U.S. is rented (Bigelow et al. 2016). These factors are synergistic in prohibiting the implementation of cover crops. It is unreasonable to expect a farmer to implement sustainable practices to their economic detriment, particularly when the land they farm is rented. This gives a farmer little stake in the long term quality and viability of agricultural land. While poorly utilized, cover cropping is observed to be an extremely effective tool for nutrient mitigation. rye, in particular, has been documented reducing nitrate leaching by up to 93% (Lee et al. 2014). Additionally, cover crops are generally more effective than other nutrient mitigation strategies. Comparatively little water leaves a field as surface runoff, rather it percolates through the soil profile. Cover crops have the potential to absorb water and nutrients within the soil profile where buffer strips cannot. While yield drag is a documented issue regarding cover crops, over time they increase cash crop yield by providing essential nutrients to the soil as they decompose (Woodruff et al. 2019). Cover crops have the potential to be an essential nutrient reduction and soil retention tool, but see challenges to implementation due to economic factors.

pennycress (*Thlaspi arvense* L.) is a fall cover crop that is generally classified as a weed. Planted after corn or soy in the fall, pennycress overwinters and generates oil producing seed in the spring that can be used as vegetable oil or biodiesel. Because of this, pennycress has the potential to act as both a fall cover crop and a cash crop (McGinn et al. 2019). However, when compared to other cover crops, such as winter camelina, pennycress is less effective at nutrient mitigation (Lee et al. 2014, Lui et al 2020). The only thing making pennycress desirable for widespread implementation over other cover crops is its ability to overcome the economic hurdle of cover crop implementation. While potentially lacking in nutrient mitigation, the use of pennycress as a cover crop could still provide a vital ecosystem service that has the rare potential to receive widespread implementation due to its position as an oilseed crop (Johnson et al 2017, McGinn et al. 2019). pennycress is being rapidly modified by both universities and corporations through gene editing techniques. The likelihood for the widespread implementation of pennycress is relatively high and because of this the way its ecosystem effects must be better understood. Therefore, to better assess the effectiveness of pennycress as a cover crop and its interactions with abiotic factors of the environment it is essential to develop a better understanding of pennycress and genetically modified strains of pennycress.

The purpose of this study is to develop a better understanding of the way pennycress interacts with its environment by analyzing the rate at which it loses biomass and N in relation to other cover crops. This was done by comparing the patterns of biomass and nitrogen loss from cereal rye, annual rye, wild type pennycress, and *AOP2* pennycress in two different soil types representative of central Illinois. This is essential to understand as patterns of biomass and nitrogen loss from cover crops may have considerable impacts on the growth of cash crops. We aim to create a better understanding of the rate at which these cover crops decompose while also comparing patterns of decomposition between soil type. While cereal rye and annual rye are known to be effective cover crops, they may not decompose and release nitrogen at a rate that is beneficial to cash crop production (Sievers and Cook 2018). The decomposition of pennycress is much less understood. While pennycress may not be as effective at nutrient uptake as cereal rye or annual rye, it has far more potential for implementation due to its ability to create profit for farmers. Should it decompose and release nitrogen to the soil at a rate that is beneficial to primary cash crop production, it may see more widespread adoption as a cover crop. The objective of this study is to compare the rates of decomposition and nitrogen loss between rye and pennycress such that a better understanding of the ecosystem effects of cover crops can be attained. The aim is to create a better understanding of a plant that may receive widespread implementation as an essential nutrient mitigation strategy in the agricultural midwest, we have no intention of selling or promoting a product.

METHODS

This study was conducted at the Illinois State University farm in Lexington, Illinois. Prior to the study, a scythe and hand clippers were used to harvest aboveground biomass from annual rye, cereal rye, and wild type pennycress at the ISU farm. Aboveground biomass for gene-edited *AOP2* pennycress was received from Western Illinois University. It was not possible to collect biomass from all species at one location because we were unable to obtain seed for *AOP2* pennycress. The *AOP2* gene was edited out of pennycress using CRISPR to stop production of glucosinolates. Glucosinolates act as a defense mechanism from herbivory in most plants of the order Brassicales ([Sønderby](https://www-sciencedirect-com.libproxy.lib.ilstu.edu/science/article/pii/S1360138510000312#!) et al 2010). In large amounts, glucosinolates can be toxic to both humans and herbivorous vertebrates. To make pennycress better suited to a cash crop market, its ability to produce glucosinolates was removed. Once aboveground biomass for all cover crops was collected, it was dried in a 50°C drying oven for 7 days. Following this, the biomass roughly chopped into small pieces using both a paper cutter and food processor. 20g of the cut biomass was placed into labeled ANKOM R1020 10x20cm mesh forage bags. This served as the initial weight. In total, 396 forage bags were made so that each crop had 99 individual bags. Bags were secured closed using loop lock labels and anchored into the soil surface using bamboo cooking skewers with a small amount of dirt placed on top using a shovel.

A blocked study design was used accounting for pseudoreplication. Blocks were assigned by soil type with bags placed in one of two prevalent soil types found in central Illinois as determined using the USGS soil web survey (USDA 2019). Saybrook stil loam (soil series 721A) was the most prevalent soil with a combination of Drummer and Elpaso silty clay loam (soil series 145B2) being the next most prevalent. Saybrook series soils are formed in loess or in other silty material and in underlying loamy till on plains and moraines. Slope ranges from 0 to 20 percent. Mean annual precipitation for Saybrook is about 914 mm (36 in) and mean annual temp is about 10°C (National Cooperative Soil Survey). Drummer and Elpaso soils are very similar with Drummer series soils being deep and poorly drained and formed in loess or other silty material and in the underlying loamy stratified outwash on nearly level or depressional parts of outwash plains, stream terraces, and till plains. The Drummer series also has an average annual rainfall of 940 mm and an average annual temperature of 11°C (National Cooperative Soil Survey). Elpaso soils are very deep, poor draining soil formed in loess and glacial till on uplands. Slope ranges from 0 to 2 percent. Mean annual precipitation for Elpaso is about 940 mm (37 in) and mean annual temperature is about 11°C (National Cooperative Soil Survey).

Forage bags were placed on the soil surface in between rows of corn with a small amount of dirt placed on top using a shovel. To account for pseudoreplication, samples were placed in 10 rows of corn, with 9 groupings of 4 bags in each row. One group from each row was sampled at sample dates with the group sampled determined by a random number generator. An equal number of samples was collected from each soil type on collection dates. T = 0 samples were taken to the field and immediately brought back to the lab to account for handling loss. Sample dates were chosen such that later dates had more time in between sampling than earlier dates and dates were scheduled as close to harvest as possible. Sampling took place 0, 7, 14, 21, 28, 36, 45, 54, 63, and 72 days from initial placement. Once forage bags were collected, they were washed using a standard method of dunking and scrubbing the bags in DI water to remove dirt from inside and outside the bags. Following this, the bags were air dried under fans and in a 50°C drying oven until bag weight stabilized. They were then weighed for dry mass after which biomass was removed from the bags and ground using a Wiley Mill to be sent to XXXXX for Nitrogen analysis.

Statistics:

To estimate decomposition rate (k) we used untransformed percent mass losses of material to fit a nonlinear regression model (1)

M(t) = M(0)e-kt + ε (1)

for each of the replicates of mass loss (Adair et al. 2010). To determine differences in the rate of decomposition values of k we used a 2 way fixed ANOVA with soil type and cover crop species as fixed factors using the R package car (Fox and Weisberg 2019). Followup pairwise comparisons were made using the emmeans package (Lenth 2019) using a Bonferroni correction. All data manipulation and statistical analyses were completed using R version 4.1.1 Kick Things ((R Development Core Team 2021) with the package Tidyverse (Whickham et al. 2019).

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Fig 1. Map of Illinois State University Farm in Lexington, Illinois with soil series superimposed on top. The white dots represent each block in the study with the bottom dot representing

Saybrook soils and the top dot representing Drummer and Elpaso soils.

RESULTS

Anova Table (Type III tests)

Response: k

Sum Sq Df F value Pr(>F)

(Intercept) 0.00066149 1 214.9633 9.430e-16 \*\*\*

sp 0.00013413 3 14.5296 3.812e-06 \*\*\*

soil\_block 0.00011065 1 35.9568 1.097e-06 \*\*\*

sp:soil\_block 0.00004654 3 5.0412 0.005665 \*\*

Residuals 0.00009847 32

Post F Test

sp soil\_block emmean SE df lower.CL upper.CL .group

gm\_pc 1 0.00453 0.000785 32 0.00223 0.00682 a

gm\_pc 2 0.00560 0.000785 32 0.00330 0.00789 ab

pc 1 0.00720 0.000785 32 0.00491 0.00950 abc

pc 2 0.00906 0.000785 32 0.00676 0.01135 bcd

cr 1 0.00940 0.000785 32 0.00710 0.01169 cd

ar 1 0.01150 0.000785 32 0.00921 0.01380 de

cr 2 0.01327 0.000785 32 0.01098 0.01557 e

ar 2 0.01815 0.000785 32 0.01586 0.02045 f

Confidence level used: 0.95

Conf-level adjustment: bonferroni method for 8 estimates

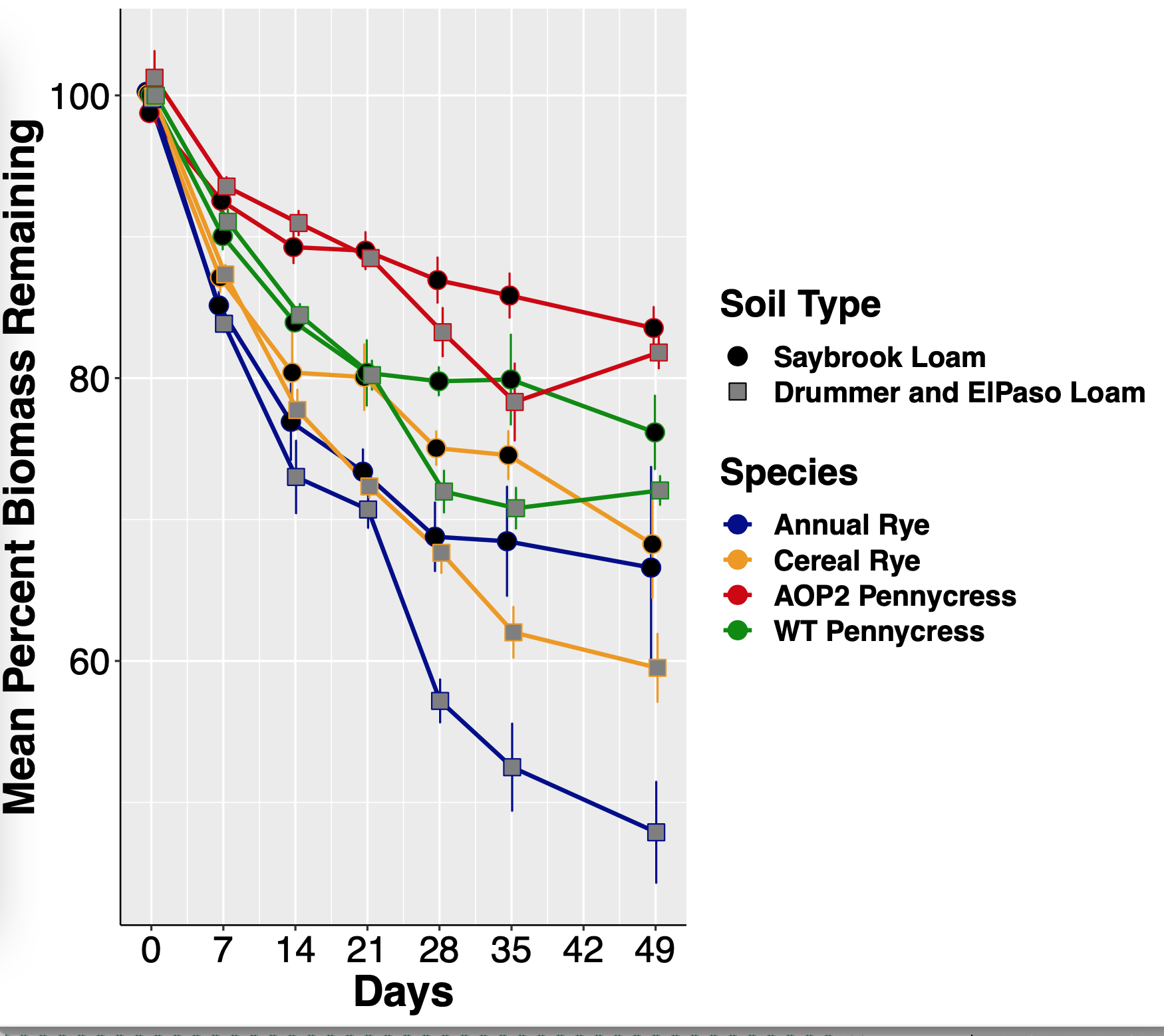
P value adjustment: bonferroni method for 28 tests

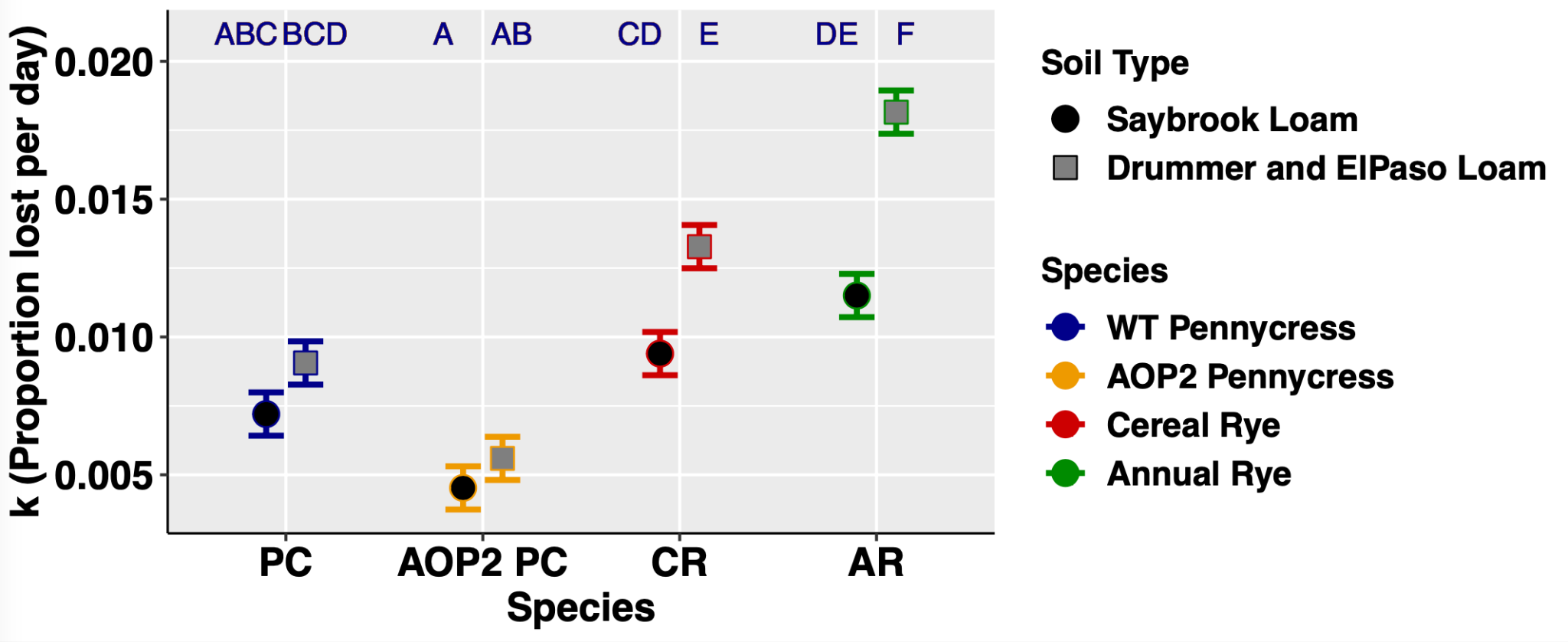
significance level used: alpha = 0.05

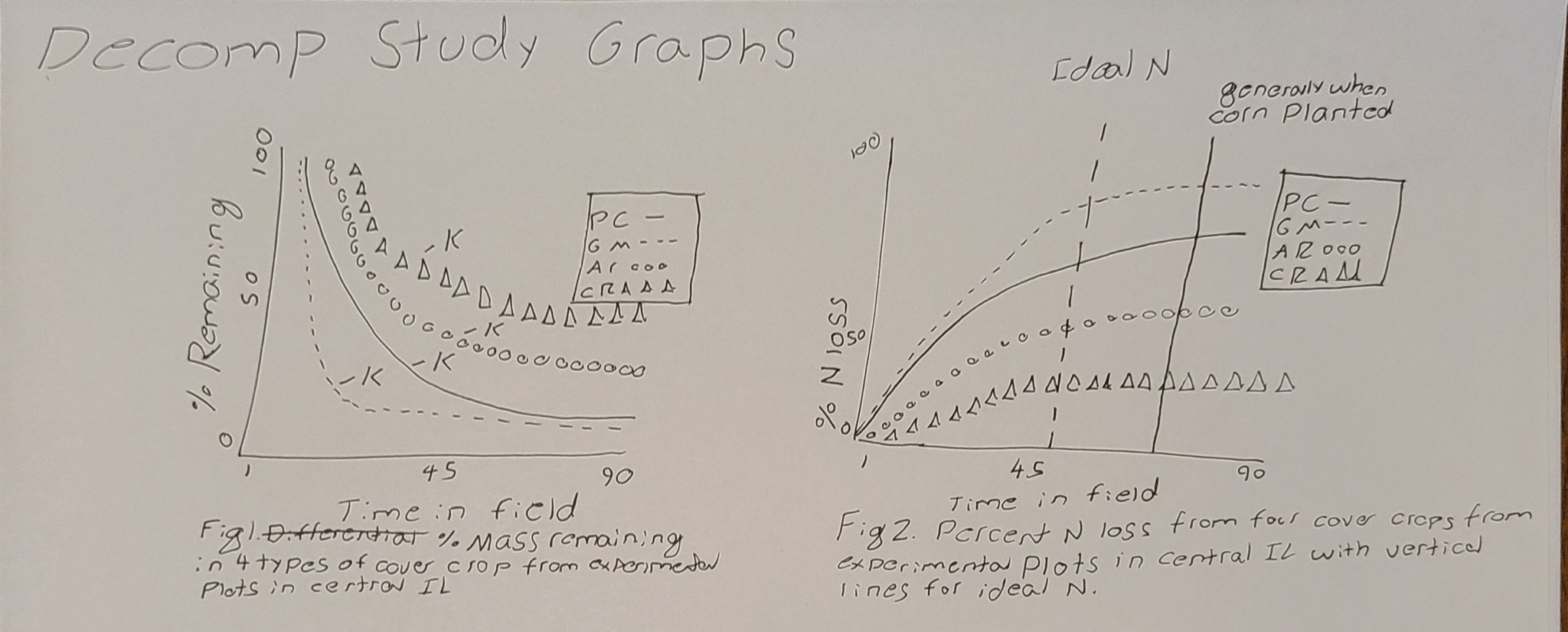
NOTE: Compact letter displays can be misleading

because they show NON-findings rather than findings.

Consider using 'pairs()', 'pwpp()', or 'pwpm()' instead.







DISCUSSION

We hypothesized that annual rye, cereal rye, wilt type pennycress, and *AOP2* pennycress would decompose exponentially in regards to both biomass and N loss as labile compounds decomposed quickly leaving recalcitrant molecules to decompose more slowly (Dhakal et al 2020). Understanding rates of decomposition is essential due to its influence on yield. Rapid decomposition may leave nutrients to leach out of the soil too early and slow decomposition may not allow nutrients essential to cash crop growth to become available at the right time (Zupwayi et al. 2004). Regardless of decomposition, cover crops provide diversity to agricultural systems which can be highly beneficial to soil conditions (McDaniel et al 2014). There are myriad factors affecting the decomposition of cover crops, however providing a generalized model for decomposition and N loss can inform the implementation of cover crops as a sustainable agricultural practice. Rates of decomposition and N loss do not provide the full picture of the ecosystem effects of cover cropping, but they do provide a strong basis for developing a robust understanding of cash cover crops.

This part is reporting the results and accepting or rejecting the hypotheses that we stated earlier so I can’t write too much of it right now.

* K factors
* P-values
* gross/net loss
* When do we need the nutrients in the field for them to be most effective for production?

In order to reduce nutrient pollution both in the midwest and the Gulf of Mexico, the midwest must be more proactive in implementing sustainable agricultural practices. While N application improves productivity, in excess it is detrimental to soil quality and the ability of soils to retain N. In addition to polluting surface water, surplus N application can reduce soil fertility and crop yield (Mukesh et al 2019). The mechanisms for N loss from soil are volatilization, denitrification, leaching, runoff, and erosion (Dinnes et al 2002, Lamb et al 2014, Mukesh et al 2019, Paramasivam et al 2019). Cover crops provide a solution to both soil and nutrient loss. Aboveground and belowground biomass from cover crops can reduce soil loss from erosion and uptake surplus nutrients that would otherwise flow to the gulf (Da Baets et al 2011, Batteny and Grimser 2000). Cover crops are an effective nutrient mitigation strategy for which there is a strong need throughout the agricultural midwest. Highlighting this is the annual hypoxic zone created in the gulf of Mexico caused almost exclusively by poor farming practices in the midwest.

Eutrophication at the gulf has been a known issue for decades with the agricultural productivity of the midwest being the primary cause (Harknik et al 2017). However, no legislation has been enacted to provide sustainable solutions to this eutrophication. This is indicative of an inability to rely on top-down solutions to eutrophication. Therefore, bottom-up solutions must be relied upon. Cover cropping has the potential to be an effective bottom-up solution and while known to be effective as a sustainable farming practice, it does not provide income to farmers (Cecchin 2021). This is a major hurdle in the implementation of cover crops and often serves as the primary factor hindering their implementation. Another primary concern of farmers is that of yield drag following the termination of cover crops. Cash crop rotations, subsurface drainage, and tillage are known to significantly affect corn yield, and there is concern that cover cropping will decrease yield, adding further complexity to the goal of maximizing yield (Acharya et al 2019). Cover crops uptake soil nutrients and soil moisture disallowing their use by cash crops which can have a negative effect on yield. Furthermore, there is the possibility that cover crops can introduce pathogens or pests to corn and soy. Research suggests that under cool and wet conditions cereal rye can reduce corn seedling growth and increase incidence of corn root disease with hairy vetch and canola having similar, but somewhat lessened impacts (Schneck et al 2017). Further research regarding yield drag produces similar results. Over multiple studies, various cover crops seem to have somewhat negative or negligible effects on corn and soy yield (Acharya et al 2020, Bavougian et al 2019, Bashyal et al 2019, Ott et al 2019, Raper et al 2019). However, over time, cover crops are documented as increasing corn yield which provides a strong argument for the long term implementation of cover crops (Adler et al 2020, Cai et al 2019). Literature focused on field pennycress produces a wide array of results with both negative and positive effects documented (Bishop and Nelson 2019, Johnson et al 2015). Because cover crops increase yield over long time frames, there is a strong argument for the long term establishment of cover crops and cover cropping culture. It is difficult, however, to advertise a nutrient mitigation solution that will cause initial yield drag. This makes the rates of decomposition and nutrient loss for cover crops essential to understand as this could have a large bearing on cover crop implementation. Should a cover crop release nutrients in a way that is beneficial to cash crop production, this could serve as a boon for the implementation of cash crops as a sustainable farming practice.

While potential solutions to nutrient pollution include both edge-of-field and in-field practices, we argue that in-field practices show more promise for nutrient mitigation. Edge-of-field practices generally refer to buffer strips composed of natrive prairie plants or tree cover and can be highly reliant upon field geography (Dabney et al 2006). In certain situations buffer strips can be highly effective, but in tiled agricultural systems there is relatively little surface runoff as most of the water hitting a field percolates through the soil and into drainage tiles. Because of this, buffer strips may not have a considerable impact on nutrient mitigation. In these systems, in-field practices such as cover crops can be highly effective. Cover cropping ensures that excess nutrients are pulled out of the soil before they move through the tiles and into streams or drainage ditches. The lack of reliance on field geography for cover cropping to be successful ensures that they are an effective solution to nutrient pollution.

Cover crops are effective at nutrient mitigation and necessary for improving the health of ecosystems locally and globally (Moore et al. 2020). However, there are many challenges for implementing cover crops on a scale large enough to reduce nutrient pollution to acceptable levels. Perhaps the primary driver for the lack of cover crop implementation is that cover crops are generally not economical (Cechin et al 2021). Pennycress, however, is an oilseed producing crop that can be used as a cash cover crop, giving it the potential to be effective at nutrient mitigation while also being economical. We are not interested in the development of pennycress as a product and view the myriad conflicts of interest in its development and research with strong distaste. However, pennycress has the potential to provide positive benefits to globally important ecosystems and as such we are interested in nutrient mitigation and the ecosystem effects of this crop. Understanding how pennycress decomposes and releases N in relation to genetically modified strains of pennycress and rye is essential to understanding both its potential as a cover crop and its ecosystem effects. Farming practices need to change in order to ensure the stability of the Earth to support further generations of human life. Cover crops are a sustainable farming practice that is known to be effective at nutrient mitigation and with new genetic modification can be an economical solution as well. By furthering our understanding of novel strains of pennycress there is the potential to implement this sustainable practice on a wide scale.

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