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Cover Crop Mixture Diversity and Function

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COVER CROP MIXTURE DIVERSITY AND FUNCTION

by

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A DISSERTATION

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COVER CROP MIXTURE DIVERSITY AND FUNCTION

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The objective of this study was to evaluate the effect of cover crop mixture diversity on cover crop function. Specifically, this study evaluated the effect of cover crop species and functional richness on aboveground biomass productivity, weed suppression, soil nutrient retention, soil microbial community characteristics, and performance stability. Twenty to forty cover crop treatments were replicated three to four times at eleven sites across southeastern Nebraska using a pool of eighteen species representing three cover crop species each from six pre-defined functional groups: cool-season grasses, cool-season legumes, cool-season brassicas, warm-season grasses, warm-season legumes, and warm-season broadleaves. Each species was planted in monoculture and the most diverse treatment contained all eighteen species. Remaining treatments represented intermediate levels of cover crop species and functional richness. Cover crop planting dates ranged from late July to late September with both cover crop and weed aboveground biomass being sampled prior to winterkill. Soil samples were taken in the following spring and analyzed for soil extractable nitrate, phosphorus, potassium, sulfate, and chloride as well as extracted for fatty acid methyl esters to characterize soil microbial biomass and community structure. Performance stability was assessed by evaluating the variability in cover crop biomass for each treatment across plots within each site. While increasing cover crop mixture diversity increased average aboveground biomass productivity, I argue that this was simply the result of the average performance of the monocultures being drawn down by low yielding species rather than due to niche complementarity or increased resource use efficiency. Furthermore, while increases in cover crop mixture diversity were often correlated with increases in weed suppression, increases in soil nutrient retention, increases in soil microbial biomass, alterations in soil microbial community structure, and increases in performance stability, I argue that this was a result of diversity co-varying with aboveground biomass, and that differences in aboveground biomass rather than differences in diversity drove the differences observed in weed suppression, nutrient retention, soil microbial community characteristics, and stability. The results of this study contradict many popular hypotheses regarding the relationship between plant mixture diversity and function.

Dedication

To my husband, Robert, with thanks.

Acknowledgements

I have been incredibly fortunate in the people I have been able to work with during my time at the University of Nebraska. For these people and to these people I would like to express my heartfelt thanks. First and foremost I want to thank my major advisor, Dr. John Lindquist. Thank you for being both understanding and expectant and supporting me throughout this process. Thank you also for all your thoughtful feedback on my work and for giving me all sorts of room to roam with this project. I could not have asked for a better adviser. Appreciation also goes to the other members of my supervisory committee— Dr. Rhae Drijber, Dr. Charles Francis, and Dr. Leon Higley. Thank you for helping me to think about things in new ways and for your wonderful senses of humor. I've enjoyed learning from all of you very much.

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Chapter 1 - Introduction

Cover crops have long been used for a wide variety of functions including adding organic matter, suppressing weeds, decreasing nutrient leaching, and stimulating soil biota. Recently, however, there has been increased interest in the use highly diverse mixtures of cover crops. While it's been asserted that mixing cover crops does everything from increasing biomass productivity, to increasing weed suppression, to enhancing nutrient retention, to fostering soil health through stimulating increased soil biota, to buffering against environmental variability, there is actually little empirical evidence to support these claims. It has been proposed that the many functions of cover crops are only improved with the use of more cover crop species, but these claims are based less on empirical evidence and based more on an intuition about diversity that prevails in both the fields of agriculture and ecology. The overarching objective of this project was to determine the effects of increasing cover crop mixture diversity on cover crop function. Cover crops are used for various functions and the goal of this project was to see if increasing cover crop diversity could be used as a tool to positively manage these functions.

In Chapter 2, I evaluate whether increasing cover crop mixture diversity increases average aboveground biomass. While increasing diversity did in many cases increase average aboveground biomass, I question the traditional interpretation of this kind of observation as evidence of the niche complementarity or increased resource use efficiency of mixtures. I propose an alternate interpretation of this kind of observation—simply that monocultures tend to have lower average productivity because of the presence of low yielding monocultures.

In Chapter 3, I evaluate whether increasing cover crop mixture species richness increases weed suppression. While increased cover crop species richness was associated with increased weed suppression, once I controlled for the positive relationship between cover crop aboveground biomass and weed suppression, there was no observable effect of cover crop mixture species richness on weed suppression. In Chapter 3, I also evaluate whether grass cover crops are more suppressive of grass weeds than broadleaf cover crops and vice versa. I find no evidence that weeds are more suppressed by cover crops that are "more similar" to them.

In Chapter 4, I evaluate whether increasing cover crop mixture species richness increases soil nutrient retention. Specifically, I look at the concentrations and distributions of soil extractable soil nitrate, phosphorus, potassium, sulfate, and chloride in the upper 60 cm of soil. I find evidence that cover cropping increases the retention of the relatively mobile soil nutrients—nitrate, sulfate, and chloride—in the upper portions of the soil profile and that these increases are mediated by cover crop biomass productivity, but no evidence that increasing cover crop mixture species richness increases soil nutrient retention.

In Chapter 5, I evaluate the effect of increasing cover crop mixture diversity, as measured by both cover crop mixture species and functional richness, on soil microbial biomass and community structure. I find that cover cropping increases soil microbial biomass and that these increases are positively related to cover crop aboveground biomass but not to cover crop mixture diversity. Similarly, I find that while cover cropping alters soil microbial community structure and that these alterations are predictably affected by cover crop biomass, they are not predictably affected by cover crop mixture diversity.

In Chapter 6, I evaluate the effect of increasing cover crop mixture diversity, as measured by both cover crop species and functional richness, on plot-to-plot aboveground biomass variability. While increasing cover crop mixture diversity was correlated with decreases in variability and therefore increases in stability, I find that this relationship is driven by variations in aboveground biomass. More productive treatments tended to experience less variability. Once I accounted for the effect of productivity on variability, I found only marginal effects of cover crop mixture diversity on stability.

In each of the chapters, I challenge previous workers in the field on a variety of issues, but my primary criticism is that many previous workers simply misinterpret correlation as causation. That is, they interpret the correlation of diversity with various metrics of weed suppression, soil nutrient retention, soil microbial biomass, and stability as indications of diversity affecting these metrics. I found in this study and others, however, that once we take into account variations in biomass productivity between treatments, these apparent relationships between diversity and function disappeared. This is to say that productive monocultures were just as good at suppressing weeds, retaining nutrients, increasing soil microbial biomass, and performing stably as productive mixtures and the reason that it sometimes appears that mixtures are better at doing these things is because productive mixtures are being compared to unproductive monocultures.

In Chapter 7, I attempt to consolidate each of these separate findings into a single, coherent narrative for cover crops and place this narrative into the broader context of existing diversity research.

Chapter 2 - Cover crop mixture diversity and productivity

ABSTRACT

The diversity-productivity hypothesis proposes that increasing plant diversity increases average biomass productivity. The goal of this study was to test this hypothesis in the context of cover crop mixtures. Eighteen species of cover crops were used in this study representing six functional groups—cool-season grasses, cool-season legumes, cool-season brassicas, warm-season grasses, warm-season legumes, and warm-season broadleaves. Twenty to forty treatments reflecting varying levels of species and functional richness were planted at eleven sites across southeastern Nebraska. Cover crop treatments ranged from containing one species to containing all eighteen species. Planting dates ranged from July 19 to September 20. Species specific aboveground biomass measurements were taken prior to winterkill. Four sites were not sampled due to issues of cover crop establishment. Of the seven sites sampled, there was little evidence that increasing species richness without increasing functional richness increased average productivity. However, increasing functional richness had a marked positive effect on average aboveground biomass. The implications of this, however, are more mathematical than practical. The lower yielding legumes lowered the average productivity of the low functional richness category as compared to the high functional richness category where the higher yielding grasses and brassicas compensated for the low production of the legumes. In terms of practical cover crop management, there was no evidence of any mixture out-yielding the highest yielding monoculture at each site. While the diversity-productivity hypothesis was supported—this study suggests a rather simple, mathematical mechanism by which increasing diversity can increase average productivity.

INTRODUCTION

The diversity-productivity hypothesis proposes that greater diversity should lead, on average, to greater total biomass productivity (Tilman, 2001). The most common argument is that a single species leaves resources unexploited that another species might be able to come in and exploit—i.e., that more diverse systems are more productive due to increased niche complementarity or resource use efficiency. While many authors have observed a positive correlation between manipulated diversity and average productivity, the interpretation of these results as evidence of niche complementarity is contested (rev. deLaplante and Picasso, 2011).

Despite the controversy surrounding the diversity-productivity hypothesis, the idea that increased diversity equates increased average productivity has been entrenched in many fields as fact—particularly in agriculture. It's not uncommon, for example, to read in the agricultural sciences that mixed cropping is associated with increased productivity (e.g., Anil et al., 1998; Ćupina et al., 2011; Lithourgidis et al., 2011; Seran and Brintha, 2010).[1] In one telling line, Ćupina et al. (2011) states that intercropping is "a practical application of ecological principles based on biodiversity." Thus, it's clear that at least by some agricultural scientists, the diversity-productivity hypothesis is taken as proven principle instead of as an unproven hypothesis.

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[1] It should be noted that other workers in the field are much more cautious with their language. Rather than saying that intercropping increases productivity, they say that carefully designed mixtures have the potential to increase productivity—a subtle, but important difference (e.g., Francis, 1989; Malezieux et al., 2009).

Putting aside empirical evidence in favor of or against the hypothesis for a moment, why might we expect diversity to be positively related to productivity? The reasons given in both the ecological and agricultural sciences are the same—though slightly different language is favored. In the field of ecology, it's not uncommon to hear reference to "niche differentiation", "partitioning", and "complementarity" (Lawton et al., 1998). In the field of agriculture, it's more common to hear reference to "resource use efficiency" (iterum, Anil et al., 1998; Ćupina et al., 2011; Lithourgidis et al., 2011; Seran and Brintha, 2010). The logic, however, is the same—that each species has different resource needs and different resource acquisition abilities. A monoculture therefore leaves some resources unexploited that another species might be able to exploit—e.g., through its differential root or canopy architecture. Thus, plant mixtures should have the potential to out produce plant monocultures because mixtures should be able to more fully exploit available resources (Vandermeer et al., 2002). That is, mixing plants should be able to raise the ceiling on biomass productivity reached by plant monocultures. *This, however, is a different conclusion than increasing diversity increases average productivity.*

Interestingly, the logic commonly used to argue in favor of the diversity-productivity hypothesis, when taken to its logical conclusion, supports a different hypothesis. Increasing *average* productivity is not the same as increasing the *ceiling* on productivity. According to the logic of niche complementarity, increasing diversity shouldn't necessarily increase average productivity. Rather it should increase the ceiling on productivity. This disconnect between the theoretical underpinnings of the diversity-productivity hypothesis and the theoretical conclusions of the diversity-productivity hypothesis indicates two things. First, it indicates that we should be testing the theory of niche complementarity by testing whether increasing mixture diversity raises the ceiling on productivity rather than average productivity. Second, it indicates that niche complementarity is not a sensible explanation for the diversity-productivity hypothesis as stated, or the necessary conclusion to be drawn from diversity-productivity observations.

The original objective of this study was to test the diversity-productivity hypothesis in the context of cover crop mixtures. The result has been to technically support the diversity-productivity hypothesis—i.e., to show an increase in average productivity with increased diversity—but to also demonstrate some flaws with the traditional interpretation of this as evidence of niche complementarity.

The primary and most unrelenting criticism of diversity-productivity research has been that the experimental designs of these studies are such that more productive species are more likely to be present in the higher levels of diversity. This effect has been variously called the "sampling effect", the "selection effect", the "sampling bias", and the "selection bias" with the results of a study with such an effect being called "experimental artifact" (Aarsen, 1997; Huston, 1997; Huston et al., 2000; Wardle, 1999). In this study I demonstrate that (1) even without sampling bias, positive diversity-productivity relationships can still persist, and (2) even so, niche complementarity need not be invoked as the driving mechanism. Rather, a simple mathematical explanation exists to explain the observation—specifically, the average productivity of lower levels of diversity is drawn down by low yielding species while the average productivity of higher levels of diversity is not drawn down to the same degree because high yielding species make up for low yielding species in mixture.

Before delving into the study, however, I want to briefly address the topic of facilitation. Facilitation effects between species are also cited as a possible mechanism for positive diversity-productivity relationships. However, I think listing this as an additional mechanism to niche complementarity confuses the issue. Facilitative effects are a mechanism by which a particular species might enhance the growth of another. This is more of a pair-wise interaction rather than the effect of diversity itself. Certainly the likelihood of this pair-wise interaction increases with increasing diversity, but if we use that logic to support the diversity-productivity hypothesis, how do we resolve that with the fact that increasing diversity also increases the likelihood of negative pair-wise interactions such as allelopathic interactions? It's my opinion that we cannot point to positive pair-wise interactions in our justification of the diversity-productivity hypothesis without also acknowledging the potential for negative pair-wise interactions. Furthermore, while the likelihood of including particular pair-wise interactions increases with increasing diversity, the relative effect of that pair-wise interaction is decreased or diluted with increasing diversity.

As yet another source of potential confusion, facilitation is sometimes regarded as a kind of complementarity (e.g., Cardinale et al., 2007). Here, however, I have chosen to draw a sharp distinction between complementarity, which I regard as the result of individual species having differing requirements, and facilitation, which I regard as the ability of one species or individual to modify the environment favorably for another (Callaway, 1995; Callaway and Pugnaire, 2007; Scherer-Lorenzen, 2008). It's within this framework and with these definitions that I evaluate the positive diversity-productivity relationships observed in this study.

MATERIALS AND METHODS

Research sites

This study was conducted at eleven sites across southeastern Nebraska. Cover crops were planted at a variety of points in a variety of crop rotations (Table 2-1). With the exception of site numbers 1 and 4, which were irrigated, all other sites were rain-fed.

Table 2-1. Study locations, planting dates, planting conditions, and sampling dates.

Site	Location	Planting date	Planting conditions	Sampling date
1	40°24'60"N 99° 2'60"W	7/19/2013	Wheat stubble	NA
2	40°58'25"N 97°59'15"W	8/10/2013	Barley stubble	NA
3	41°40'15"N 96°33'45"W	8/31/2013	Wheat stubble (disked)	10/31/2013
4	41°10'20"N 96°27'30"W	9/10/2013	Soybeans (R5)	11/9/2013
5	41°40'10"N 96°33'50"W	9/12/2013	Soybeans (R7)	11/7/2013
6	41°40'20"N 96°34'5"W	9/12/2013	Corn (R6)	NA
7	40°58'10"N 97°59'50"W	9/14/2013	Soybeans (R6)	11/14/2013
8	41°19'45"N 96°16'55"W	9/19/2013	Corn stubble (disked)	11/8/2013
9	40°19'5"N 98°35'45"W	9/20/2013	Corn (R6)	NA
10	41°40'20"N 96°33'40"W	7/20/2014	Wheat stubble (disked)	9/27/2014
11	40°51'5"N 96°28'10"W	7/23/2014	Wheat stubble	10/14-15/2014

Experimental design

Treatments

The study was started in 2013 with twenty treatments representing monocultures and mixtures of nine species—barley, oat, wheat, Austrian winter pea, red clover, yellow sweetclover, radish, rapeseed, and turnip (Table 2-2). The nine species were selected to represent three functional groups—cool-season grasses, cool-season legumes, and brassicas. Note that the cool-season grasses used were all spring varieties, which winterkilled along with the legumes and brassicas.

Table 2-2. Summary of cover crop treatments for 2013.

	No.	Functional group(s)	Treatment	No. of species	No. of groups
	1	-	No cover	0	0
Monocultures	2	Cool-season grasses (CG)	Barley (BAR)	1	1
	3		Oats (OAT)	1	1
	4		Wheat (WHT)	1	1
	5	Cool-season legumes (CL)	Austrian winter pea (PEA)	1	1
	6		Red clover (RED)	1	1
	7		Yellow sweetclover (YEL)	1	1
	8	Cool-season brassicas (CB)	Radish (RAD)	1	1
	9		Rapeseed (RAPE)	1	1
	10		Turnip (TURN)	1	1
Mixtures	11	CG	BAR + OAT + WHT	3	1
	12	CL	PEA + RED + YEL	3	1
	13	CB	RAD + RAPE + TURN	3	1
	14	CG + CL	BAR + OAT + WHT + PEA + RED + YEL	6	2
	15	CG + CB	BAR + OAT + WHT + RAD + RAPE + TURN	6	2
	16	CL + CB	PEA + RED + YEL + RAD + RAPE + TURN	6	2
	17	CG + CL + CB	All 9 cool-season species	9	3
	18	CG + CL + CB	BAR + PEA + RAD	3	3
	19		OAT + RED + RAPE	3	3
	20		WHT + YEL + TURN	3	3

Treatment 1 was a no cover control. Treatments 2-10 were all the species included in the study grown in monoculture.

Treatment 11 was a mixture of all three cool-season grasses, while treatments 12 and 13 were mixtures of all three cool-season legumes and brassicas, respectively. These treatments served to evaluate the effect of increasing species diversity without increasing functional diversity.

Treatment 14 combined the cool-season grasses with the cool-season legumes, and treatment 15 combined the cool-season legumes with the brassicas, while treatment

16 combined the cool-season grasses with the brassicas. The reasons for these treatments were to be able to observe any specific interactions between functional groups and to have a level of functional diversity intermediate between the prior treatments and treatment 17. Treatment 17 combined all nine species used.

Treatments 18-20 were random combinations of one cool-season grass, one cool-season legume, and one brassica. These treatments were included as a way to evaluate the effect of increasing species richness without increasing functional richness and as a way to evaluate the effect of increasing functional richness without increasing species richness. These last three treatments were designed so that each of the nine species was present in one of the three treatments. This was to avoid the issue of sampling bias.

In designing all of the treatments used, a point was made to make sure that each species was equally represented at each level of species and functional richness to address the issue of sampling bias.

In 2014, the study was expanded to include an additional 20 treatments (Table 2-3). Of these additional treatments, treatments 21-39 represented warm-season analogues of treatments 2-20. That is, warm-season grasses, warm-season legumes, and warm-season broadleaves were used instead of the cool-season grasses, cool-season legumes, and brassicas. The species used were proso millet, sorghum sudangrass, teff, chickpea, cowpea, sunn hemp, buckwheat, safflower, and sunflower.

Treatment 40 was a combination of the original nine cool-season species and these nine warm-season species. This treatment wasn’t used in the analysis for this particular study into biomass productivity but it was used in the chapters that follow and has been included here for the sake of comprehensiveness.

Table 2-3. Summary of cover crop treatments added in 2014.

	No.	Functional group(s)	Treatment	No. of species	No. of groups
Monocultures	21	Warm-season grasses (WG)	Proso millet (PROSO)	1	1
	22		Sorghum sudangrass (SORG)	1	1
	23		Teff (TEFF)	1	1
	24	Warm-season legumes (WL)	Chickpea (CHICK)	1	1
	25		Cowpea (COW)	1	1
	26		Sunn hemp (SUNN)	1	1
	27	Warm-season broadleaves (CB)	Buckwheat (BUCK)	1	1
	28		Safflower (SAFF)	1	1
	29		Sunflower (SUNF)	1	1
Mixtures	30	WG	PROSO + SORG + TEFF	3	1
	31	WL	CHICK + COW + SUNN	3	1
	32	WB	BUCK + SAFF + SUNF	3	1
	33	WG + WL	PROSO + SORG + TEFF + CHICK + COW + SUNN	6	2
	34	WG + WB	PROSO + SORG + TEFF + BUCK + SAFF + SUNF	6	2
	35	WL + WB	CHICK + COW + SUNN+ BUCK + SAFF + SUNF	6	2
	36	WG + WL + WB	All 9 warm-season species	9	3
	37	WG + WL + WB	PROSO + CHICK + BUCK	3	3
	38		SORG + COW + SAFF	3	3
	39		TEFF + SUNN + SUNF	3	3
	40	CG + CL + CB + WG + WL + WB	All 18 species	18	6

Seeding rates

Seeding rates for the different cover crops in monoculture are presented in Table 2-4. These seeding rates were based on recommended broadcast rates (Clark, 2007). Cover crop mixture seeding rates were proportional to the rates used in monoculture. For example, in a three species mix, each species was planted at one-third the full rate listed. The seeding rates for the brassica species were reduced in the second year of this study as it was deemed the original seeding rate was higher than necessary to achieve maximum biomass.

Table 2-4. Seeding rates used for each cover crop species in monoculture.

Functional group	Species	Scientific Name	Seeding rate ($g \cdot m^2$)
CS-G	Barley	*Hordeum vulgare* L.	16.8
	Oats	*Avena sativa* L.	16.8
	Wheat	*Triticum aestivum* L.	16.8
CS-L	Austrian winter peas	*Pisum sativum* L. ssp. s*ativum* var. *arvense*	11.2
	Red clover	*Trifolium pratense* L.	1.7
	Yellow blossom sweetclover	*Melilotus officinalis* (L.) Lam.	1.7
CS-B	Radish	*Raphanus sativus* L.	1.7*
	Rapeseed	*Brassica napus* L. var. *napus*	1.7*
	Turnip	*Brassica rapa* L. var. *rapa*	1.7*
WS-G	Proso millet	*Panicum miliaceum* L.	2.8
	Sorghum sudangrass	*Sorghum bicolor* (L.) Moench x *Sorghum bicolor* (L.) Moench var. sudanese	5.6
	Teff	*Eragrostis tef* (Zuccagni) Trotter	0.6
W-SL	Chickpea	*Cicer arietinum* L.	16.8
	Cowpea	*Vigna unguiculata* (L.) Walp.	11.2
	Sunn hemp	*Crotalaria juncea* L.	5.6
WS-B	Buckwheat	*Fagopyrum esculentum* Moench	11.2
	Safflower	*Carthamus tinctorius* L.	2.8
	Sunflower	*Helianthus annuus* L.	0.6

*Seeding rate decreased to 1.1 $g \cdot m^2$ in 2014.

Treatment establishment

Treatments were arranged in a randomized complete block design with four replications at each site with the exception of site 11, which had only three replications for each treatment due to space constraints. Plots were 5 x 10 m—though these dimensions varied slightly to accommodate corn and soybean row spacings at sites 4, 5, 6, 7, and 9. Treatments were hand broadcast into a variety of field conditions—after small grains harvest, after corn harvest, and into maturing corn and soybeans. In some instances harvested small grain fields were disked prior to cover crop establishment, in others the cover crop seeds were broadcast into standing stubble (Table 2-1). Field management decisions were left up to each cooperating farmer.

Plant sampling

Cover crop aboveground biomass was harvested approximately two months after planting. Vegetation was sampled using two randomly placed quadrats (0.18 m^2) in each plot for site 3 and one randomly placed quadrat in each plot for the rest of the sites harvested. Cover crops were cut at the soil surface, separated by species and dried at 55°C for 7 days and weighed to determine dry biomass. Where present, weed aboveground biomass was also sampled at this time. This data is presented in Chapter 3, which discusses cover crop diversity and invasibility.

Data analysis

The typical approach in ecology to analyzing the relationship between productivity and diversity is to regress productivity against diversity—treating a significant positive trending regression as evidence of the diversity-productivity hypothesis. I have avoided this approach because I think that there’s a more straightforward way to test the hypotheses. Moreover, the use of regression analysis in this context can be misleading—an issue I will explore further in the discussion section of this chapter. Instead, the approach taken here has been to calculate estimates of the “effect size” of increasing species and functional richness and then to test whether or not that effect is equal to zero using a simple one-sample t-test.

Species richness

To separate the effects of species richness from the effects of functional richness, I asked the question: “Does increasing species richness without increasing functional richness increase aboveground biomass?” I approached this question in two ways: (1) by tripling species richness within each functional group, and (2) by tripling the species richness of already functionally diverse mixtures. In the first case, for example, I took the aboveground biomass of the mixture that contained all cool-season grasses (treatment 11) and subtracted the average performance of the constituent monocultures (treatments 2, 3, and 4—barley, oats, and wheat, respectively). I then divided the difference by the monoculture average and multiplied by 100 to express the effect size as a percent.

$$\text{Effect size (\%)} = \frac{B_{3\ species\ mix} - \overline{B}_{mono}}{\overline{B}_{mono}} * 100$$

In the second case, I compared the aboveground biomass of treatments 18, 19, and 20—these treatments each contained one cool-season grass, one cool-season legume, and one brassica ($\overline{B}_{18,19,20}$)—to treatment 17, which contained three cool-season grasses, three cool-season legumes, and three brassicas (B_{17}).

$$\text{Effect size (\%)} = \frac{B_{17} - \overline{B}_{18,19,20}}{\overline{B}_{18,19,20}} * 100$$

Functional richness

To determine the effect of increasing functional richness alone, I held species richness constant at three species and increased functional richness from one functional group to three. That is I compared the aboveground biomass of treatments 11, 12, and 13 to treatments 18, 19, and 20.

$$\text{Effect size (\%)} = \frac{\overline{B}_{18,19,20} - \overline{B}_{11,12,13}}{\overline{B}_{11,12,13}} * 100$$

Species richness and functional richness combined

The effect of increasing species richness and functional richness simultaneously was tested by taking the aboveground biomass of the nine-species mixture (i.e., treatment 17) and subtracting the average aboveground biomass of those nine species (i.e., treatments 2-10), and then dividing by that average production of the monocultures.

$$\text{Effect size (\%)} = \frac{B_{17} - \overline{B}_{2\text{-}10}}{\overline{B}_{2\text{-}10}} * 100$$

Performing these calculations across multiple blocks and sites results in multiple estimates of effect size. To these approximately normal populations of estimates, I applied simple one-sample t-tests to determine the effects of (1) increasing species richness alone, (2) increasing functional richness alone, and (3) increasing species and functional richness together. In addition to reporting a simple dichotomous yes or no result of the t-test—i.e., "is there or isn't there an effect?"—I report the 50% and 95% confidence intervals (CI) of the estimates.

Due to irregularities in the warm-season species data, which will be discussed in the results, as well as the low number of repetitions of these treatments, these treatments were excluded from this analysis, though treatment summary data are provided. All statistical analyses were conducted using R 3.1.0 (R Core Team, 2014).

RESULTS

Cover crop productivity by site

Of the 11 sites planted, 4 were not harvested for cover crops. At site 1, cover crop establishment was patchy throughout the site due to wheat stubble being swathed after cover crop planting. At site 2, there was no cover crop growth due to extreme weed pressure. At sites 6 and 9 there was minimal cover crop growth (< 25 g m^{-2})—likely due to a combination of moisture, light, and heat limitations.

Of those sites that were harvested, the earlier planting dates had the greatest aboveground biomass, with negligible biomass for those sites planted after the beginning of September (Figure 2-1). This result is consistent with the observation by Odhiambo and Bomke (2001) that late planted fall cover crops produce less dry matter than earlier planted fall cover crops. While there were likely other factors also affecting the variation between sites observed in Figure 2-1 (e.g., moisture, light, planting conditions), successful fall cover crop establishment is much more likely with an earlier planting time—dry matter production being significantly correlated with growing degree days (Brennan and Boyd, 2012).

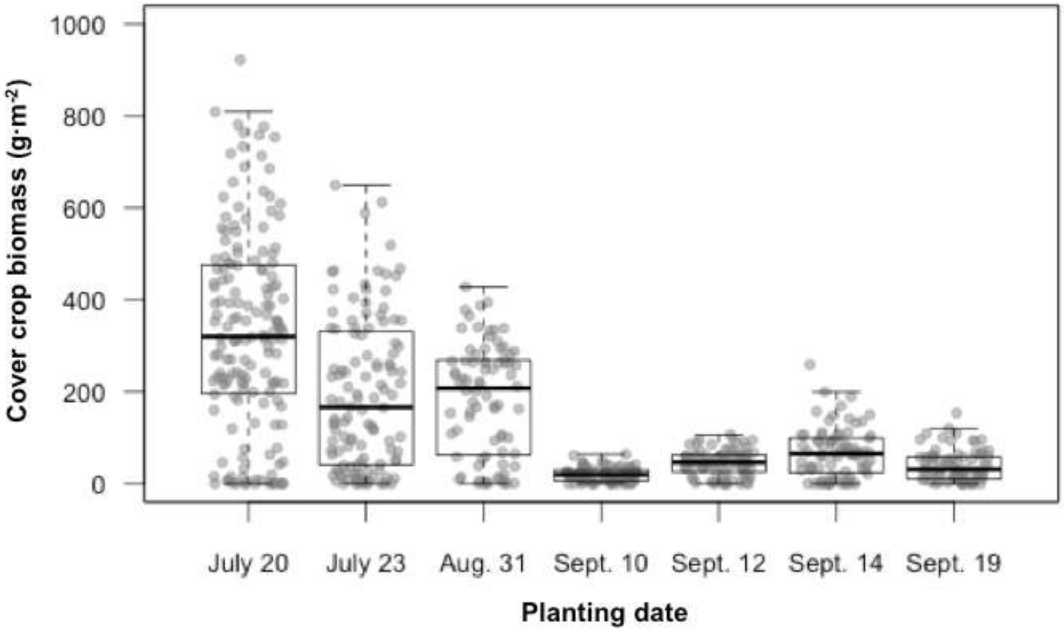

Figure 2-1. Boxplots of cover crop aboveground biomass for treatments #2-20 by planting date overlaid by individual data points which are jittered horizontally to aid in viewing. Three data points with biomass beyond 1000 g m^{-2} not shown but included in the creation of the boxplots. Note that planting dates are not temporally equidistant.

Cover crop productivity by treatment

Cover crop productivity by treatment varied widely across sites but a few patterns were consistent across all sites.

Monocultures

With regard to the cool-season species, the grasses and brassicas almost always out-produced the slower growing legumes (Figure 2-2; Figure 2-3). The best performing cool-season grass and brassica varied between sites. However, of the cool-season legumes, winter pea consistently out-produced red clover and yellow blossom sweetclover, which produced negligible aboveground biomass at all sites.

As with the cool-season grasses and cool-season legumes, warm-season grasses tended to out-produce the warm-season legumes (Figure 2-4). The warm-season legumes, tended to produce more than their cool-season counterparts, with the exception of chickpea, which performed poorly at both sites. As for the warm-season broadleaves, buckwheat was consistently one of the most productive warm-season species, safflower was generally one of the least productive, and sunflower productivity was highly inconsistent across the two sites. This is likely due to deer having grazed on the sunflower plants at site 11 but not site 3 prior to sampling.

Sampling at sites 3 and 11 happened after some of the warm-season species began to shed their foliage, leading the aboveground biomass values for some of the warm-season species to be less than they might have been had plant biomass been sampled earlier in the season. Figure 2-5 shows how warm-season species were beginning to senesce at cover crop harvest—brown colored plots—while cool-season species were continuing to grow—green colored plots. These irregularities in the warm-season species should be kept in mind when considering their biomass performance.

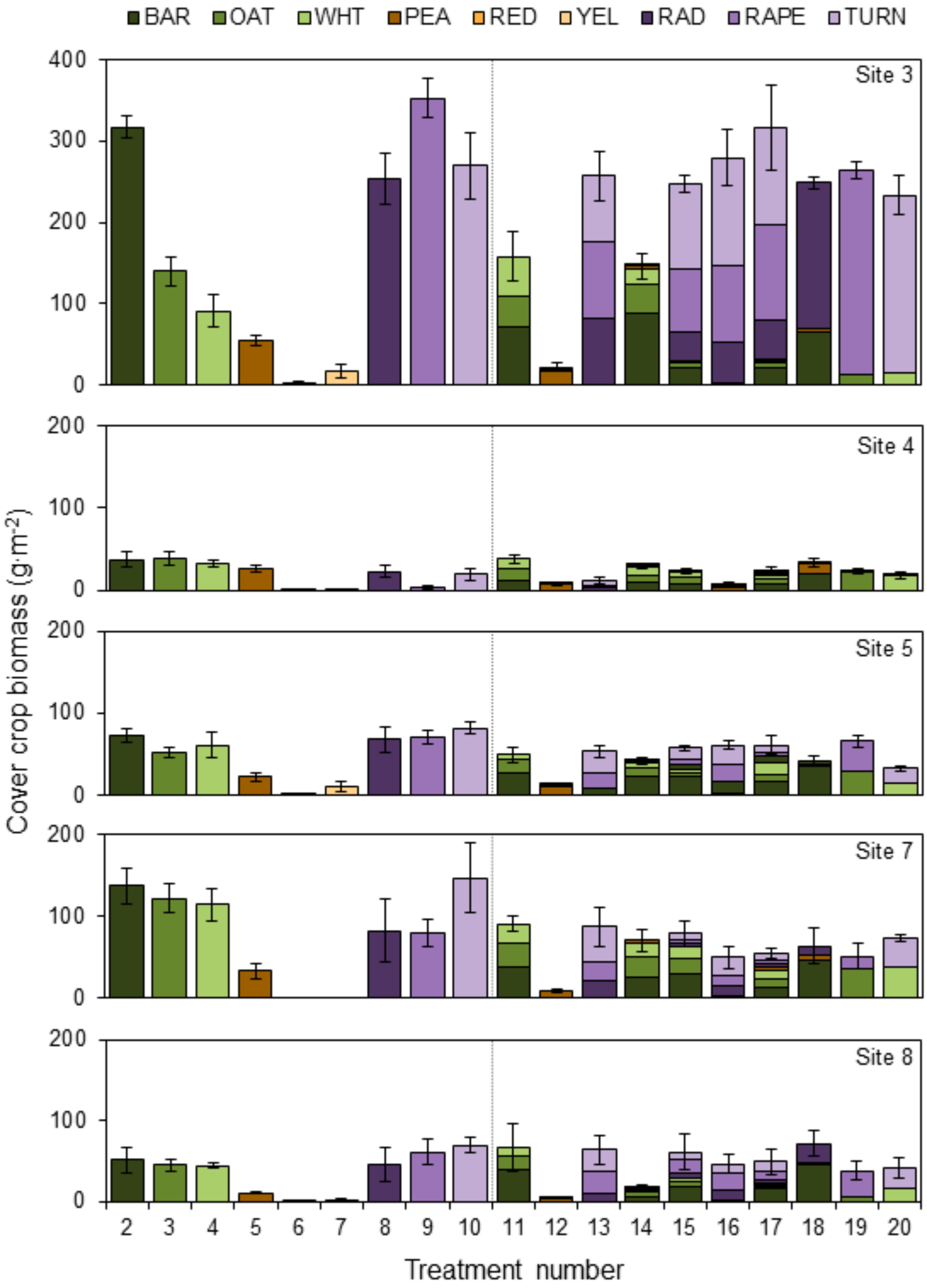

Figure 2-2. Species specific cover crop biomass (±SEM) for treatments 2-20 by site for 2013. The vertical dotted line separates monoculture (left) from mixtures (right).

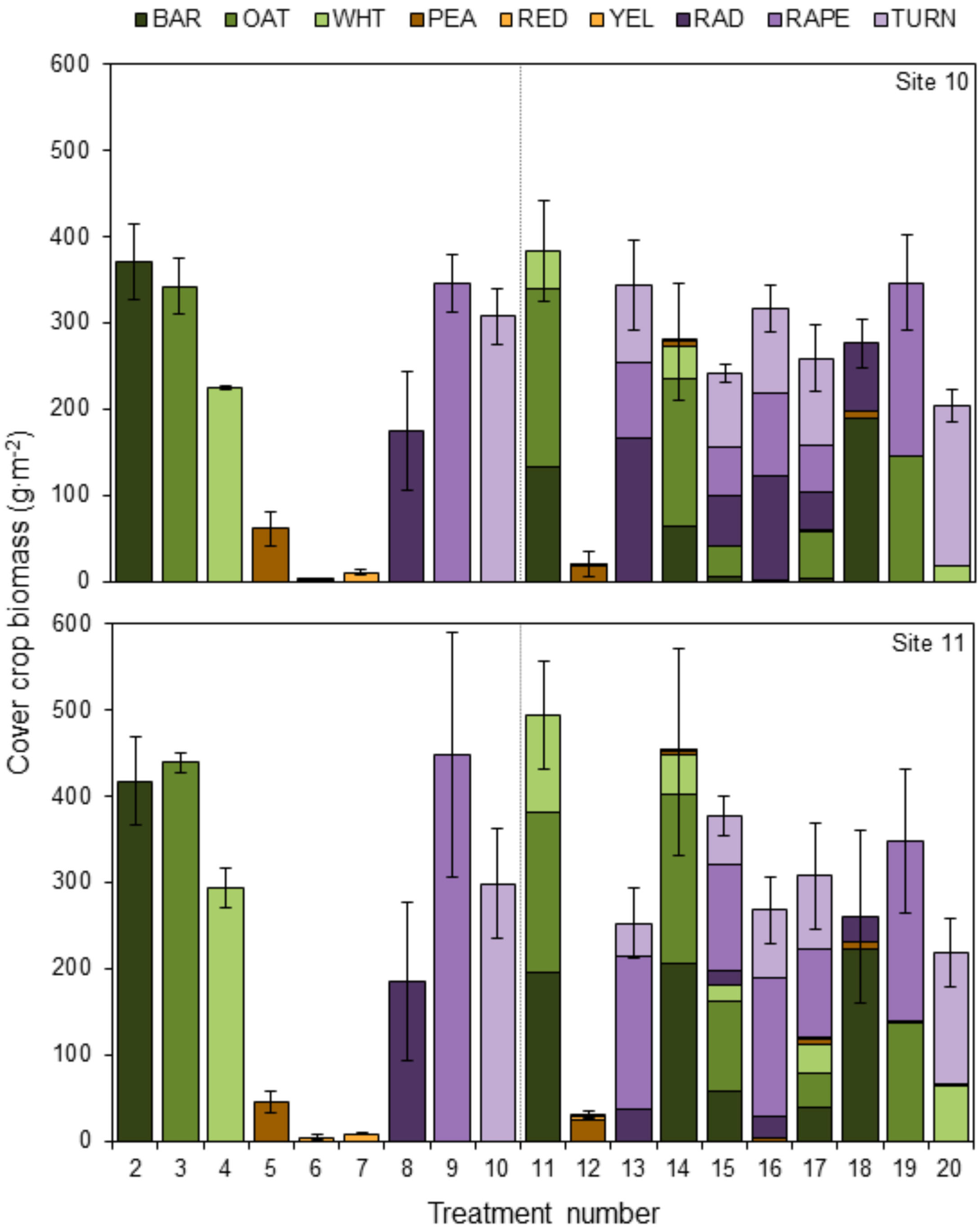

Figure 2-3. Species specific cover crop biomass (±SEM) for treatments 2-20 by site for 2014. The vertical dotted line separates monoculture (left) from mixtures (right). One extreme outlier (1156 g·m^2) for rapeseed was omitted from the bar chart for Site 11.

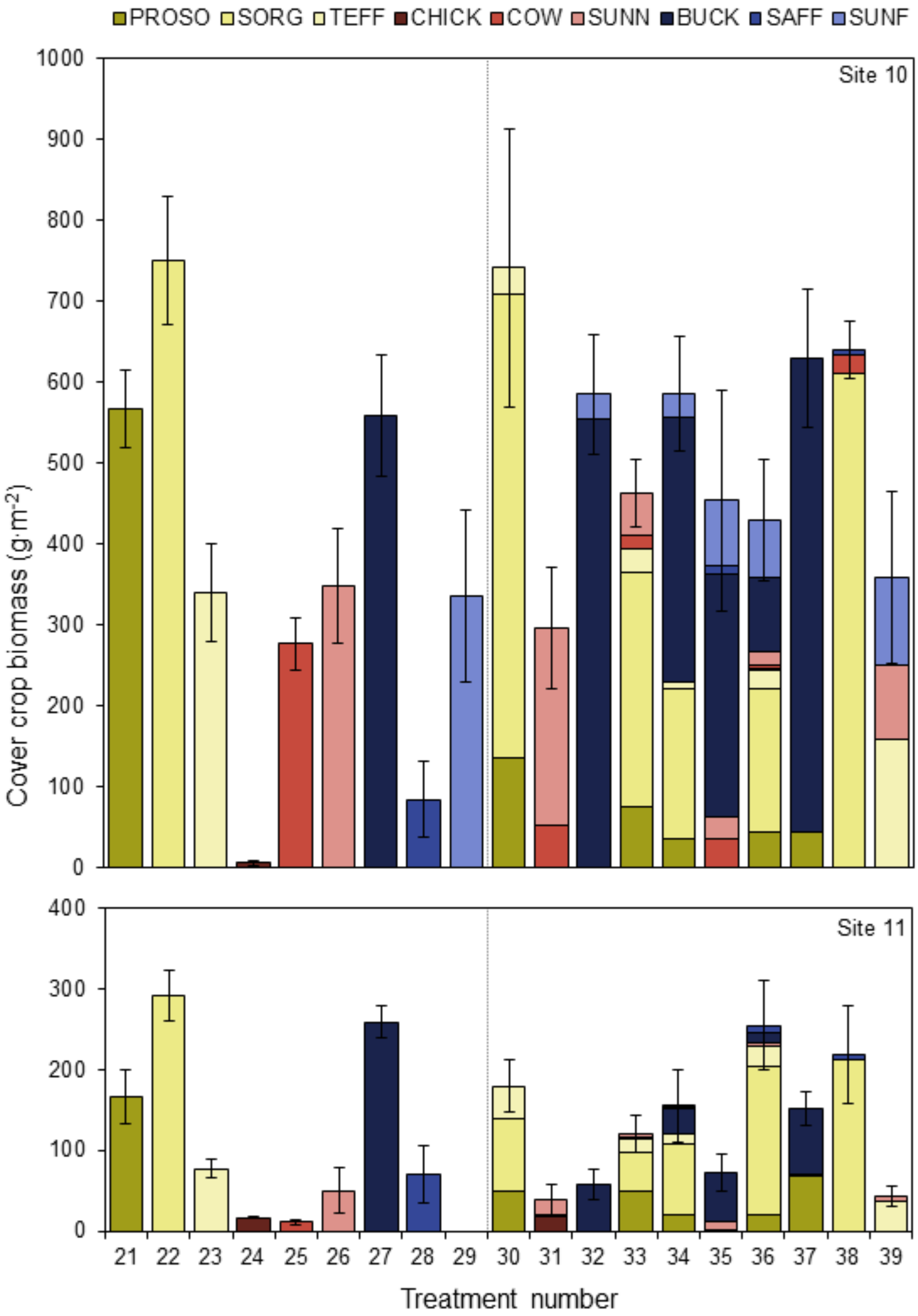

Figure 2-4. Species specific cover crop biomass (±SEM) for treatments 21-39 by site for 2014. The vertical dotted line separates monocultures (left) from mixtures (right).

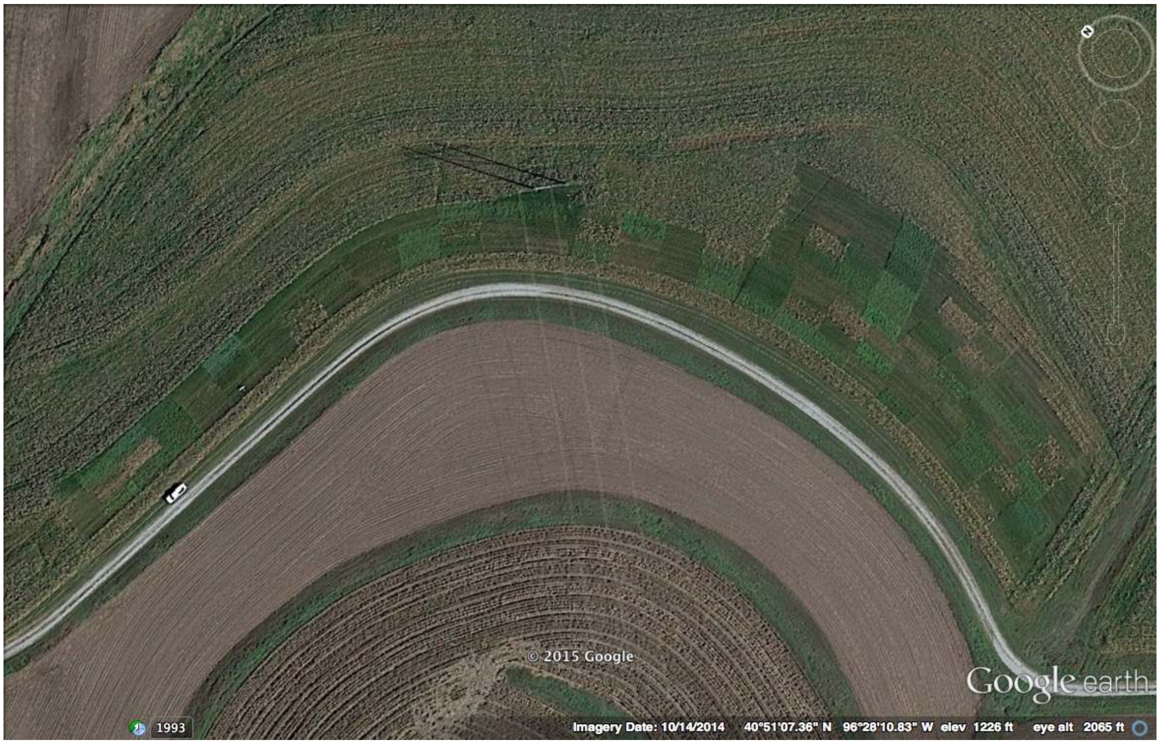

Figure 2-5. Google Earth aerial imagery of Site 11 at time of cover crop harvest.

Mixtures

The cool-season mixtures tended to be dominated by brassicas when present. The warm-season mixtures tended to be dominated by sorghum sudangrass and buckwheat when present. A species performance in monoculture was fairly predictive of its performance in mixture (i.e., high yielding species in monoculture continued to be high yielding in mixture and low yielding species in monoculture continued to be low yielding species in mixture). At no site did any mixture significantly out-yield the most productive monoculture.

Cover crop diversity and productivity

Increasing species richness while holding functional richness constant did not significantly increase average aboveground biomass (mean effect size = 2.3%, 95% C.I.

= [-7.2, 11.9%], N = 107, *p*-value = 0.65). However, increasing functional richness while holding species richness constant, increased aboveground biomass by an average of 28.6%, and increasing both functional and species richness simultaneously increased aboveground biomass by an average of 27.9% (Figure 2-6).

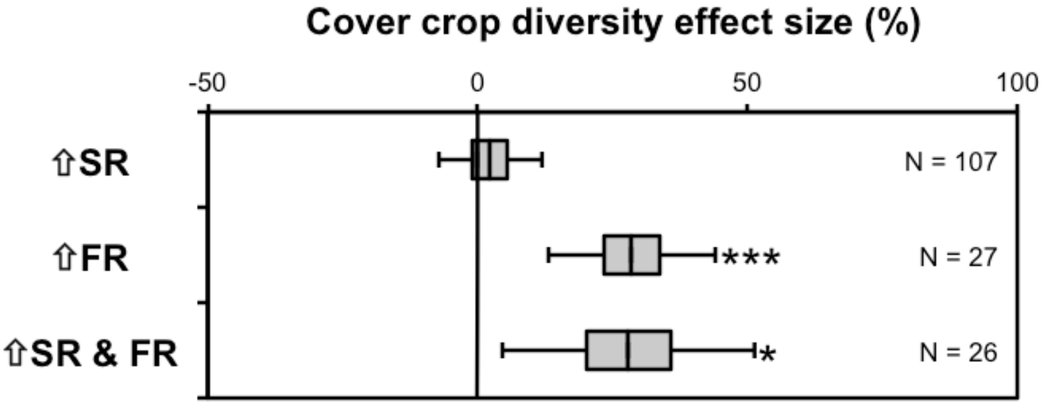

Figure 2-6. Mean effect size of increasing cover crop diversity on cover crop productivity—specifically the effects of increasing species richness (⇧SR), increasing functional richness (⇧FR), and increasing both species and functional richness simultaneously (⇧SR & FR). Boxes and bars represent 50% and 95% confidence intervals, respectively. N = number of observations for each estimate. One observation is missing from the ⇧SR & FR category. Asterisks indicate *p*-value for the following test—H_0: $\mu = 0$; H_a: $\mu \neq 0$. *P*-value > 0.05 (no asterisk); < 0.05(*); < 0.01(**); < 0.001(***).

DISCUSSION

Increasing plant mixture diversity, particularly functional richness, was associated with increased average aboveground biomass. This is consistent with previous findings using manipulated grasslands and other plant mixtures (rev. Cardinale et al., 2007; rev.

Cardinale et al., 2011; Spehn et al., 2005). I argue, however, that there is no need to invoke niche complementarity or increased resource use efficiency to explain this observation. Rather, the positive effect of increasing plant mixture diversity on average productivity is easily explained by low yielding species pulling down the average at low levels of diversity but not at high levels of diversity.

Specifically, the pattern observed was simply the consequence of the average productivity of the monocultures and low functional richness category being brought down by the low yields of the legumes. In the high diversity treatments, the high yields of grasses and brassicas compensated for the low yields of legumes. This is why mixing across functional groups led to increased average productivity but not mixing within a single functional group. Mixing the grasses or the brassicas with each other did not increase average productivity because there were no low yielding species being compensated for in the mixture. Similarly, mixing the legumes together did not increase average productivity because there was no high yielding species in the mix to compensate for the low yields of the legumes.

Much attention has been paid to the difference between species and functional richness in the literature, with some authors arguing that we pay more attention to functional richness (Diaz and Cabido, 2001). I argue that the issue is not so much about increasing species versus functional richness, but about whether the species we are mixing produce markedly different amounts of biomass when planted in monoculture. I suspect the reason functional richness appears to be a driver of productivity in many studies is that plants from the same functional group tend to produce similar amounts of biomass.

Classical ecological approach: a misleading use of regression analysis

Classical diversity-productivity studies present their results by plotting average biomass productivity against a diversity metric—most often that metric is species richness. The general approach is to regress productivity against the diversity metric. The statistical significance of such a regression is then used as evidence of the positive effect of diversity on biomass production (e.g., Fornara and Tilman, 2009; Fridley, 2002; Hector et al., 1999; Klironomos, 2000; Roscher et al., 2005; Schnitzer et al., 2011; Tilman et al., 1996; Tilman et al., 1997; Tilman et al., 2001; Zhang et al., 2010). Within this generalization there are many variations. For example, sometimes instead of using species richness as the diversity metric, functional richness or phylogenetic diversity is used (e.g., Cadotte et al., 2008; Connolley et al., 2011; Hooper, 1998; Hooper and Dukes, 2004; Hooper and Vitousek, 1997). Additionally, the x- or y- metric is sometimes transformed. For example, the logarithm of species richness might be used instead of species richness itself (e.g., Loreau et al., 2001; Naeem et al., 1996; Naeem et al., 1995) or sometimes biomass productivity is log-transformed (e.g., Jiang et al., 2007). There is also variation in terms of the form of the regression that is used. While simple linear regression is quite common, it is also popular to use non-linear regression—particularly models that show a saturating effect of diversity—i.e., decreasing returns on increasing diversity. For example, exponential, logarithmic, power, and hyperbolic functions are frequently tested and used (e.g., Cardinale et al., 2006; Hooper et al., 2005; Symstad et al., 1998, Tilman et al., 1997). All of this slight variation in analysis, however, is peripheral to and distracts from the fact that regression analysis is a poor tool for the purpose of testing and understanding the diversity-productivity hypothesis. We have prematurely asked, “What is the shape of the diversity-productivity relationship?” before we have asked “Is there a diversity-productivity relationship?” and “Why is there a diversity-productivity relationship?” Moreover, the results of regression analysis are easily misinterpreted by both casual observers and scientists deeply entrenched in the subject matter. It’s easy to misinterpret plots like the ones shown in Figure 2-7 where I have analyzed the results from Site 3 much in the fashion it would have been analyzed in the field of ecology. It’s easy to think that this figure shows that increasing plant mixture diversity increases potential biomass yield, but this is not the case with the data. While there is nothing false about what has been presented in the figure, it is nevertheless misleading.

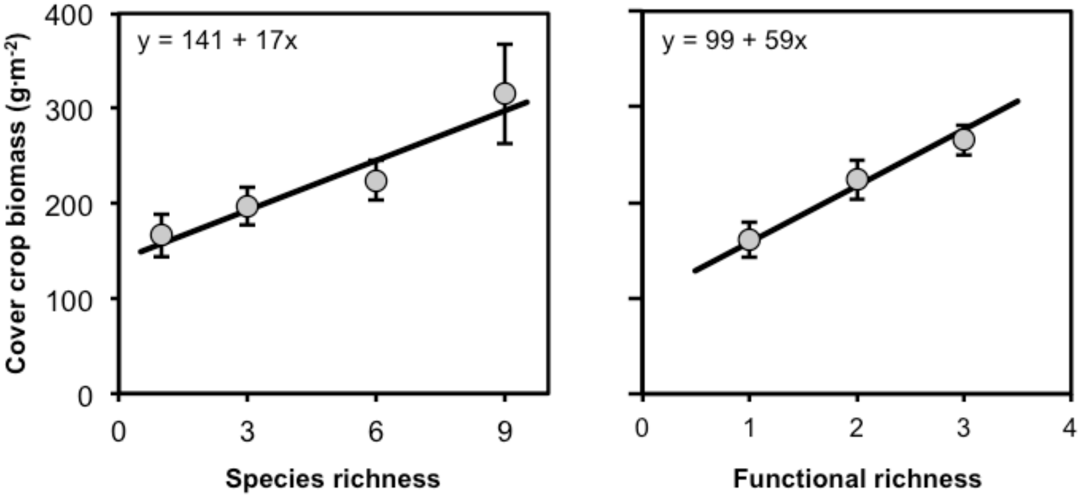

Figure 2-7. Mean cover crop biomass (±SEM) by cover crop species and functional richness for Site 3 with ordinary least squares regression lines.

Significant regressions like these are routinely used by scientists as evidence of the diversity-productivity hypothesis and furthermore of niche complementarity. My interpretation of what is happening is different, almost embarrassingly simplistic, and more apparent when we use estimated effect sizes rather than regressions to test the diversity-productivity hypothesis. Simply, when there is bare space on the ground left by a not very productive species and you add more vegetation by adding another species, you get more vegetation. While this may seem like a simple description of niche complementarity, consider the fact that we could also get more vegetation by adding more of the same species rather than a different species. For example, He et al. (2005) found that the positive relationship between diversity and productivity decreased with increasing plant density—that is, simply increasing the density of the monocultures brought the biomass up to the high levels of the mixtures. One of the untested assumptions in many plant diversity and mixed cropping studies is that the monoculture densities used are optimal, but this assumption is rarely tested.

Cover crop management conclusions

While the goal of this study was not to see if mixing cover crops could raise the ceiling on monoculture productivity, I found no evidence that simply increasing the number of species or functional groups in a cover crop mixture increased the ceiling on biomass productivity. None of the twenty-one mixtures tested outperformed the best performing monoculture of the eighteen species tested at any of the seven sites. Though there are some cases in the scientific literature where mixtures perform better than the best performing species in monoculture, in the overwhelming majority of cases they do not (Donald, 1963; Garnier et al., 1997; Picasso et al., 2008; Smith et al., 2014; Trenbath, 1974; Vandermeer et al., 2002; Wortman et al., 2012). Thus, to cover crop managers looking to maximize cover crop biomass production, I recommend picking a productive species, giving it as long of a growing season as possible as well as a weed free start. There is no indication that increasing cover crop mixture diversity will increase potential cover crop biomass productivity.

Parting thoughts regarding the plant mixture diversity and resource use efficiency

Niche theory predicts that diverse systems should have the potential to be more productive than even the most productive of monocultures by capturing a greater proportion of the available resources—but this is not what has been observed. Cardinale et al. (2006) called understanding this disconnect between theory and observation one of the foremost challenges in the diversity-productivity field. However, if a monoculture can entirely capture a single necessary resource to plant growth, such as and very often light, even if another species is able to capture additional remaining resources, that species cannot do so without that one necessary resource. Therefore the addition of species does not necessarily equate the capturing of more resources and the increasing of total biomass productivity. This idea is further explored in Chapter 3, where I discuss how a single species can be just as weed suppressive as a diverse mixture of species.

References

Aarsen, L.W. 1997. High productivity in grassland ecosystems: effects by species diversity or productive species? Oikos 80:183-184.

Anil, L., J. Park, R. H. Phipps, and F. A. Miller. 1998. Temperate intercropping of cereals for forage: A review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53:301-317.

Brennan, E. B., and N. S. Boyd. 2012. Winter Cover Crop Seeding Rate and Variety Affects during Eight Years of Organic Vegetables: I. Cover Crop Biomass Production. Agron. J. 104:684-698.

Cadotte, M. W., B. J. Cardinale, and T. H. Oakley. 2008. Evolutionary history and the effect of biodiversity on plant productivity. PNAS 105(44):17012-17017.

Callaway, R. B., and F. I. Pugnaire. 2007. Facilitation in Plant Communities. In: F. I. Pugnaire, and F. Valladares, editors, Functional Plant Ecology, 2nd ed. CRC Press, Boca Raton, FL. p. 435-455.

Callaway, R. M. 1995. Positive Interactions among Plants. Bot. Rev. 61(4):306-349.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443(26):989-992.

Cardinale, B. J., J. P. Wright, M. W. Cadotte, I. T. Carroll, A. Hector, D. S. Srivastava, M. Loreau, and J. J. Weis. 2007. Impacts of Plant Diversity on Biomass Production Increase through Time because of Species Complementarity. P. Natl. A. Sci. USA 104(46):18123-18128.

Cardinale, B. J., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I. O'Connor, and A. Gonzalez. 2011. The functional role of producer diversity in ecosystems. Am. J. Bot. 98:572-592.

Clark, A., editor. 2007. Managing Cover Crops Profitably. 3rd ed. Sustainable Agriculture Network, Beltsville, MD.

Connolly, J., M. W. Cadotte, C. Brophy, A. Dooley, J. Finn, L. Kirwan, C. Roscher, and A. Weigelt. 2011. Phylogenetically diverse grasslands are associated with pairwise interspecific processes that increase biomass. Ecology 92(7):1385-1392.

Ćupina, B., A. Mikić. F. L. Stoddard, Đ. Krstić, E. Justes, L. Bedoussac, J. Fustec, and B. Pejić. 2011. Mutual Legume Intercropping for Forage Production in Temperate Regions. In: E. Lichtfouse, editor, Genetics, Biofuels and Local Farming Systems. Sustainable Agriculture Reviews. Springer Science. Dordrecht, Netherlands. p. 347-365.

deLaplante, K. and V. Picasso. 2011. The Biodiversity-Ecosystem Function Debate in Ecology. In: D. M. Gabbay, B. Brown, P. Thagard, K. De Laplante, K. Peacock, and J. Woods, editors, Philosophy of Ecology. Handbook of the Philosophy of Science Vol. 11. Elsevier, Oxford, UK. p. 169-200.

Díaz, S. and M. Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends Ecol. Evol. 16(11)646-655.

Donald, C. M. 1963. Competition among crop and pasture plants. Adv. Agron. 15:1-118.

Fornara, D. A., and D. Tilman. 2009. Ecological Mechanisms Associated with the Positive Diversity—Productivity Relationship in an N-Limited Grassland. Ecology 90(2):408-418.

Francis, C.A. 1989. Biological efficiencies in multiple cropping systems. Adv. Agron. 42:1-42.

Fridley, J. D. 2002. Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. Oecologia 132:271-277.

Garnier, E., M-L. Navas, M. P. Austin, J. M. Lilley, and R. M. Gifford. 1997. A problem for biodiversity-productivity studies: how to compare the productivity of multispecific plant mixtures to that of monocultures. Acta Oecol. 18(6):657-670.

He, J., K. S. Wolfe-Bellin, B. Schmid, and F. A. Bazzaz. 2005. Density may alter diversity-productivity relationships in experimental plant communities. Basic Appl. Ecol. 6:505-517.

Hector, A., B. Schmid, C. Beierkuhnlein, M.C. Caldeira, M. Diemer, P.G. Dimitrakopoulos, J. Finn, H. Freitas, P.S. Giller, J. Good, R. Harris, P. Högberg, K. Huss-Danell, J. Joshi, A. Jumpponen, G. Körner, P.W. Leadley, M. Loreau, A. Minns, C.P.H. Mulder, G. O'Donovan, S.J. Otway, J.S. Pereira, A. Prinz, D.J. Read, M. Scherer-Lorenzen, E-D. Schulze, A-SD. Siamantziouras, E. Spehn, A.C. Terry, A.Y. Troumbis, F.I. Woodward, S. Yachi, J.H. Lawton. 1999. Plant diversity and productivity experiments in European grasslands. Science 286:1123-1127.

Hooper, D. U. 1998. The role of complementarity and competition in ecosystem responses to variation in plant diversity. Ecology 79(2):704-719.

Hooper, D. U., and J. S. Dukes. 2004. Overyielding among plant functional groups in a long-term experiment. Ecol. Lett. 7:94-105.

Hooper, D. U., and P. M. Vitousek. 1997. The Effects of Plant Composition and Diversity on Ecosystem Processes. Science 277:1302-1305.

Hooper, D. U., F. S. Chapin, J. J. Ewell, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. 75:3-35.

Huston, M. A., L. W. Aarssen, M. P. Austin, B. S. Cade, J. D. Fridley, E. Garnier, J. P Grime, J. Hodgson, W. K. Lauenroth, K. Thompson, J. H. Vandermeer, and D. A. Wardle. 2000. No Consistent Effect of Plant Diversity on Productivity. Science 289:1255.

Huston, M.A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110:449-460.

Jiang, X. L., W. G. Zhang, and G. Wang. 2007. Biodiversity effects on biomass production and invasion resistance in annual versus perennial plant communities. Biodivers. Conserv. 16:1983-1994.

Klironomos, J. N., J. McCune, M. Hart, and J. Neville. 2000. The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol. Lett. 3:137-141.

Lawton, J. H., S. Naeem, L. J. Thompson, A. Hector, and M. J. Crawley. 1998. Biodiversity and Ecosystem Function: Getting the Ecotron experiment in it Correct Context. Funct. Ecol. 12(5):848-852.

Lithourgidis, A. S., C. A. Dordas, C. A. Damalas, D. N. Vlachostergios. 2011. Annual intercrops: an alternative pathway for sustainable agriculture. Aust. J. Crop Sci. 5(4):396-410.

Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. 2001. Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. Science 294:804-808.

Malézieux, E., Y. Crozat, C. Dupraz, M Laurans, D. Makowski, H. Oxier-Lafontaine, B. Rapidel, S. de Tourdonnet, and M. Valantin-Morison. 2009. Mixing plant species in cropping systems: concepts, tools and models – a review. Agron. Sustain. Dev. 29:43-62.

Naeem, S., K. Håkansson, J. H. Lawton, M. J. Crawley, and L. J. Thompson. 1996. Biodiviersity and plant productivity in a model assemblage of plant species. Oikos 76:259-264.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M Woodfin. 1995. Empirical Evidence that Declining Species Diversity May Alter the Performance of Terrestrial Ecosystems. Phil. Trans. R. Soc. Lond. B 347(1321):249-262.

Odhiambo, J.J.O., and A. A. Bomke. 2001. Grass and legume cover crop effects on dry matter and nitrogen accumulation. Agron. J. 93:299-307.

Picasso, V. D., E. C. Brummer, M. Liebman, P. M. Dixon, and B. J. Wilsey. 2008. Crop Species Diversity Affects Productivity and Weed Suppression in Perennial Polycultures under Two Management Strategies. Crop Sci. 48:331-342.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Roscher, C., V. M. Temperton, M. Scherer-Lorenzen, M. Schmitz, J. Schumacher, B. Schmid, N. Buchmann, W. W. Weisser, and E-D. Schulze. 2005. Overyielding in experimental grassland communities – irrespective of species pool or spatial scale. Ecol. Lett. 8:419–429.

Scherer-Lorenzen, M. 2008. Biodiversity and Ecosystem Functioning: Basic Principles. In: W. Barthlott, K. Eduard Linsenmair, S. Porembski, editors, Biodiversity: Structure and Function, Vol. 1. EOLSS Publishers, Paris, France. p. 68-88.

Seran, T., H., and I. Brintha. 2010. Review on Maize Based Intercropping. J. Agron. 9:135-145.

Smith, R. G., L. W. Atwood, and N. D. Warren. 2014. Increased Productivity of a Cover Crop Mixture is Not Associated with Enhanced Agroecosystem Services. PLoS ONE 9(5): e97351. doi:10.1371/journal.pone.0097351

Spehn, W. M., A. Hector, J. Joshi, M. Scherer-Lorenzen, B. Schmid, E. Bazeley-White, C. Beierkuhnlein, M. C. Caldeira, M. Diember, P. G. Dimitrakopoulos, J. A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Högberg, K. Huss-Danell, A. Jumpponen, J. Koricheva, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, C. Palmborg, J. S. Pereira, A. B. Pfisterer, A. Prinz, D. J. Read, E.-D. Schulze, A.-S. D. Siamantziouras, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 2005. Ecosystem effects of biodiversity manipulations in European Grasslands. Ecol. Monogr. 75(1):37-63.

Symstad, A. J., D. Tilman, J. Willson, and J. M. H. Knops. 1998. Species Loss and Ecosystem Functioning: Effects of Species Identity and Community Composition. Oikos 81(2):389-397.

Tilman, D. 2001. Functional Diversity. In: S. A. Levin, editor, Encyclopedia of Biodiversity, Vol. 3. Academic Press, Waltham, MA. p. 109-121.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718-720.

Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The Influence of Functional Diversity and Composition on Ecosystem Processes. Science 277:1300-1302.

Tilman, D., P. B. Reich, J. Knops, D. Wedin, T. Mielke, C. Lehmann. 2001. Diversity and productivity in a long-term grassland experiment. Science 294:843-845.

Vandermeer, J., D. Lawrence, A. Symstad, and S. Hobbie. 2002. Effect of biodiversity on ecosystem functioning in managed ecosystems. In: M. Loreau, S. Naeem, and P. Inchausti, editors, Biodiversity and ecosystem functioning: Synthesis and perspectives. Oxford Univ. Press, Oxford, UK. p. 221–233.

Wardle, D.A. 1999. Is “sampling effect” a problem for experiments investigating biodiversity-ecosystem function relationships? Oikos 87:403-407.

Wortman, S. E., C. A. Francis, and J. L. Lindquist. 2012. Cover crop mixtures for the Western Corn Belt: opportunities for increased productivity and stability. Agron. J. 104:699-705.

Zhang, C-B., J. Wang, W-L Liu, S-X. Zhu, H-L. Ge, S. X. Chang, J. Chang, and Y. Ge. 2010. Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland. Ecol. Eng. 36:62-68.

Chapter 3 - Cover crop mixture diversity and weed suppression

ABSTRACT

The diversity-invasibility hypothesis proposes that increasing plant diversity increases resistance to invasion. The competition-relatedness hypothesis proposes that like species compete with each other more than unlike species. The goal of this study was to test both of these hypotheses in the context of cover crops and weed suppression. Specifically, the objectives were (1) to evaluate the effect of cover crop mixture species richness on weed suppression and (2) to evaluate the effect of cover crop type on weed suppression by weed type—specifically, to determine whether grass cover crops are better at suppressing grass weeds than broadleaf weeds and vice versa. Twenty to forty treatments were planted at three sites in southeastern Nebraska reflecting varying levels of species richness. Six grass species—barley, oats, wheat, proso millet, sorghum sudangrass, and teff—and twelve broadleaf species—Austrian winter pea, red clover, yellow blossom sweetclover, radish, rapeseed, turnip, chickpea, cowpea, sunn hemp, buckwheat, safflower, and sunflower—were used. Cover crop planting dates ranged from July 20 to August 31. Cover crop and weed aboveground biomass measurements were taken 61 to 84 days after planting. While weed suppression was correlated to cover crop species richness, this was due to cover crop species richness co-varying with cover crop aboveground biomass. Once the positive relationship between cover crop biomass and weed suppression was controlled for, there was no observable effect of cover crop species richness on weed suppression. Similarly, there was no observable effect of cover crop type on weed suppression by weed type once variations in cover crop biomass were accounted for. In essence, productive monocultures were just as weed suppressive as

productive mixtures and there was no evidence that grass cover crops were more suppressive of grass weeds or broadleaf cover crops of broadleaf weeds.

INTRODUCTION

The diversity-invasibility hypothesis proposes that species rich ecosystems are more resistant to invasion than species poor ecosystems. This hypothesis is predicated on the premise that a single species fails to fully occupy all the available niche space in an environment and that by "saturating" or "packing" all the available niche space in an environment with different resident species, we can thus pre-empt its use by invaders. Elton (1958), who is often asserted to be the first to articulate the diversity-invasibility hypothesis—which has also been variously called the biotic resistance hypothesis, the diversity-resistance hypothesis, and the ecological-resistance hypothesis—put it this way: "[invaders] will find themselves entering a highly complex community of different populations, they will search for breeding sites and find them occupied, for food that other species are already eating, for cover that other animals are sheltering in…meeting ecological resistance."

Despite the empirical evidence in favor of this hypothesis being sparse (Levine and D'Antonio, 1999; Richardson and Pyšek, 2006) and of questionable validity (Huston, 1997; Wardle, 2001), the hypothesis has nevertheless been entrenched in agriculture as conventional wisdom. Despite the lack of empirical evidence in favor of this contention in agriculture as well as ecology (rev. Moody, 1977; rev. Moody and Shetty, 1981), it is assumed by many scientists that crop mixtures are better able to capture a greater share of available resources than single species and thereby better able to suppress weeds (e.g., Altieri and Liebman, 1986; Anil et al., 1998; Buhler, 2003).

The competition-relatedness hypothesis, which has also been called the theory of limiting similarity, is traceable to Charles Darwin in the *Origin of Species*. In Darwin's words: "[a]s the species of the same genus usually have, though by no means invariably, much similarity in habits and constitution, and always in structure, the struggle will generally be more severe between them, if they come into competition with each other, than between the species of distinct genera." Darwin's examples are of different birds, mammals, and insects displacing one another. However, in its modern applications, scientists have applied this hypothesis to the management of plant invasions. That is, it has been supposed that plant species are better able to "repel" invaders similar to them because they occupy the same kind of niche.

The ability of cover crops to suppress weeds has been well established (rev. Teasdale et al., 2007), but how does cover crop mixture diversity and similarity to target weed species affect this suppressive ability? The objectives of this study were to test both the diversity-invasibility hypothesis and the competition-relatedness hypothesis in the context of cover crop mixtures and weed suppression. Specifically, our research questions were (1) does increasing cover crop mixture diversity enhance weed suppression, and (2) are grass cover crops better at suppressing grass weeds than broadleaf cover crops and vice versa?

MATERIALS AND METHODS

Research sites

Eleven research sites were established to evaluate the relationship between cover crop mixture diversity and productivity (Chapter 2). Of these eleven sites, three sites were selected to also evaluate the relationship between cover crop mixture diversity and invasibility (Table 3-1). These three sites were selected on the basis of them having both cover crop and weed species present.

Table 3-1. Location, cover crop planting date, planting conditions, and aboveground biomass sampling date of the three sites used in this study.

Site	Location	Planting date	Planting conditions	Sampling date
3	41°40'15"N 96°33'45"W	8/31/2013	Wheat stubble (disked)	10/31/2013
10	41°40'20"N 96°33'40"W	7/20/2014	Wheat stubble (disked)	9/27/2014
11	40°51'5"N 96°28'10"W	7/23/2014	Wheat stubble	10/15/2014

Experimental design

Twenty treatments representing various levels of cover crop species richness were replicated four times at site 3. Forty treatments representing various levels of cover crop species richness were replicated four times at site 10 and three times at site 11. Site 3 was planted with a pool of nine species: three grass species—barley, oats, and wheat, and six broadleaf species—Austrian winter pea, red clover, yellow blossom sweetclover, radish, and rapeseed. Sites 10 and 11 drew from a pool of eighteen species—the same nine at site 3 in addition to three more grass species—proso millet, sorghum sudangrass, and teff, and six more broadleaf species—chickpea, cowpea, sunn hemp, buckwheat, safflower, and sunflower. All of the species used were planted in monoculture as well as together in mixtures containing up to eighteen species. To address the sampling bias (Wardle 2001), each species was equally represented at each level of species richness. That is, increasing species richness did not increase the likelihood of any one of the eighteen species being included as compared to the other species. For an in depth discussion of the treatments used in this study, please refer to the experimental design section of Chapter 2.

Plant sampling

Weed and cover crop shoot aboveground biomass was sampled using two randomly placed quadrats (0.18 m^2) in each plot for site 3 and one randomly placed quadrat in each plot for sites 10 and 11. Vegetation was cut at the soil surface. Cover crop biomass was separated to species. Weed biomass was separated to species with the exception of *Amaranthus spp.* and *Setaria spp.*, which were separated to genus. After separation, samples were dried at 55°C for 7 days and weighed to determine dry biomass.

Data analysis

Percent weed biomass reduction (BR_{weed}) was calculated as:

$$BR_{weed} = \frac{W_{control} - W}{W_{control}} * 100$$

Where $w_{control}$ is the average weed biomass in the control (no cover crop) plots for each site and w is the weed biomass in the cover crop plot of interest. BR_{weed} was related to cover crop biomass (x) by an exponential equation of the form:

$$BR_{weed} = 100 - 100 * e^{-\beta_1 x}$$

Where β_1 is a fitted parameter indicating the responsiveness of weed biomass to cover crop biomass—the larger the β_1 parameter, the more responsive weed biomass is to cover crop biomass.

To assess whether or not species richness affects invasibility after controlling for the effect of cover crop biomass, a modified version of equation 2 was also fit such that:

$$BR_{weed} = 100 - 100 * e^{-\beta_1 x - \beta_2 x SR}$$

Where *SR* is cover crop species richness and β_2 is an additional fitted parameter that allows for cover crop species richness to affect the relationship between percent weed biomass reduction and cover crop biomass. Thus, the diversity-invasibility hypothesis was essentially tested by evaluating whether increasing cover crop diversity increased weed suppression of a cover crop on a per unit biomass basis (Figure 3-1).

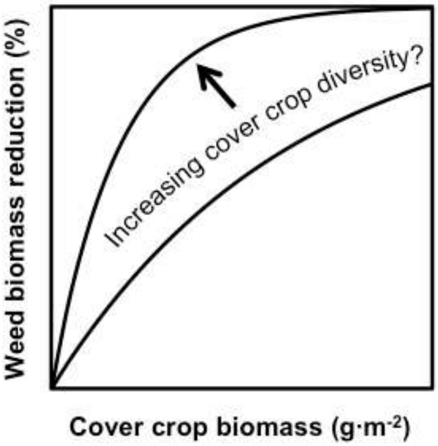

Figure 3-1. Hypothesized effect of increasing cover crop diversity—as measured by species richness—on the relationship between cover crop biomass and weed biomass reduction.

The significance of the parameter estimate β_2 and the results of an F-test, which evaluated the utility of adding the β_2 parameter to the original model, were used to draw conclusions about the impact of species richness on invasibility. Additionally, root mean squared error (RMSE) was used as an indicator of model goodness of fit to compare the models with and without the β_2 parameter.

To evaluate whether grass cover crops were more suppressive of grass weeds than broadleaf cover crops and whether broadleaf cover crops were more suppressive of broadleaf weeds than grass cover crops, the weed data were segregated into two categories—grass weeds and broadleaf weeds. Weed biomass reduction values were then calculated separately for grass weeds and broadleaf weeds. Using the same exponential model as before, weed biomass reduction for each site was modeled as a function of cover crop biomass for those cover crop treatments that were either composed of only grass species or only broadleaf species (Figure 3-2). Treatments that combined these two groups were excluded from analysis.

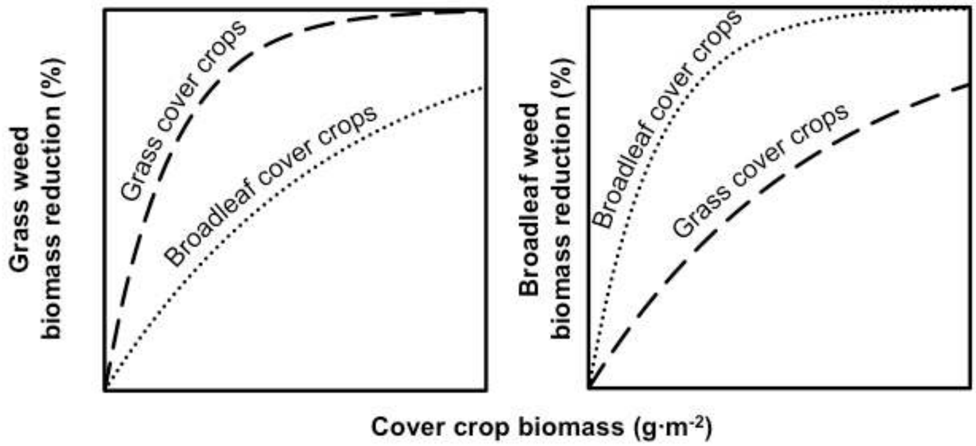

Figure 3-2. Hypothesized effect of cover crop type on the relationship between cover crop biomass and grass versus broadleaf weed biomass reduction.

Grass and broadleaf cover crop treatments were fitted to the model separately and together. The utility of separating the two categories for predicting weed loss was evaluated using an F-test. It should be noted that this procedure is equivalent to fitting a global model with both grass and broadleaf cover crop treatments and testing the utility of adding a dummy variable indicating cover crop type using an F-test.

For a more in depth discussion of how to use an F-test to compare nested models (as in Figure 3-1) and two data sets (as in Figure 3-2), refer to Motulsky and Christopoulos (2004). All statistical analyses were conducted using R 3.1.0 (R Core Team, 2014). Non-linear regression models were fit with the nls2 package by Grothendieck (2013).

RESULTS

Sown versus realized species richness

In diversity-productivity studies looking at plant mixtures, authors often have to make a decision as to whether to look at sown species richness—how many species were planted—or realized species richness—how many species were observed. Realized species richness typically correlates well to sown species richness but the deviation between realized and sown species richness tends to increase with increasing sown species richness (Figure 3-3). While I judged that realized species richness was the more appropriate metric to use here when evaluating the effect of species richness on weed suppression—as species that were planted but absent were unlikely to have an effect on weed biomass—I would like to note that using sown species richness instead of realized species richness with this data set results in the same interpretive conclusions.

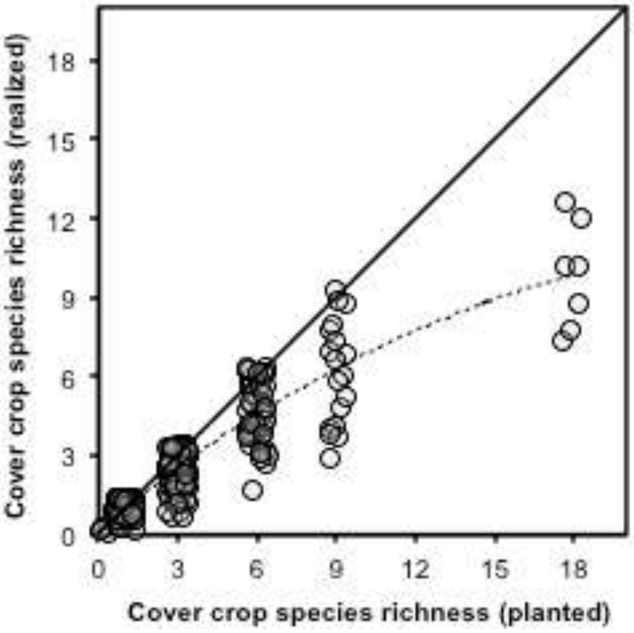

Figure 3-3. Realized cover crop species richness versus planted cover crop species richness. Points jittered along both axes for ease of viewing. Solid line indicates an idealized 1:1 relationship. Dashed line indicates LOESS curve fitted to data (α=1, λ= 2).

Classical ecological approach: mistaking correlation for causation

A typical approach to evaluating the diversity-invasibility relationship is to simply evaluate an invasion resistance metric—e.g., weed biomass reduction—as a function of a diversity metric—e.g., cover crop species richness. Any positive trending relationship is then presented as evidence in favor of the diversity-invasibility hypothesis (e.g., Figure 3-4). The problem with this approach is that it mistakes correlation with causation, and confounds the effects of diversity with the effects of biomass productivity.

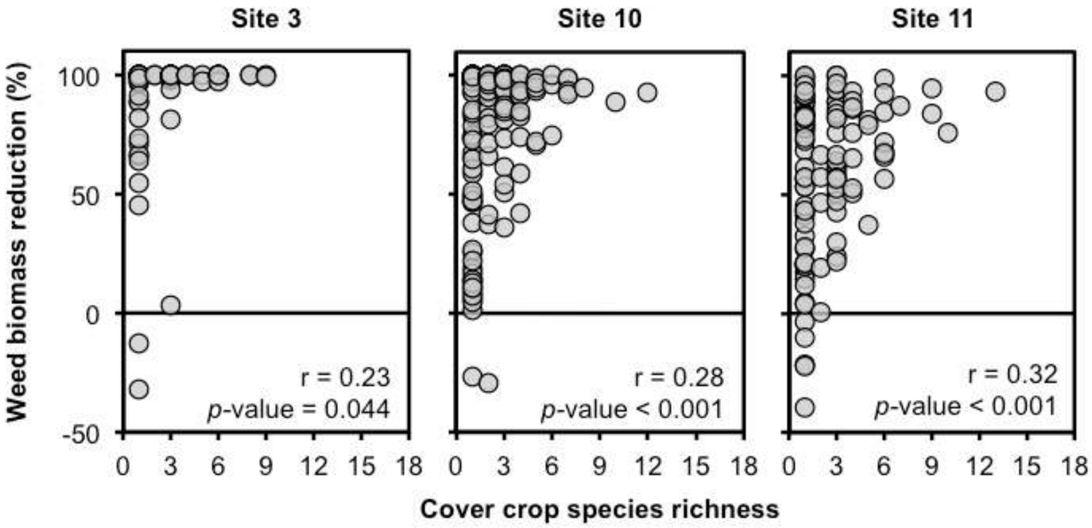

Figure 3-4. Weed biomass reduction versus cover crop species richness with Pearson correlation coefficients (r) for each site. *P*-values are for the following hypothesis test regarding the correlation coefficients—H_0: r = 0; H_a: r ≠ 0.

Plant aboveground biomass co-varies with species richness in this study as well as most other diversity-invasibility studies (Figure 3-5). Thus, it's quite possible that the correlation we see between weed suppression and species richness is due to cover crop biomass rather than species richness. To determine whether or not species richness had an effect on weed suppression beyond its relationship with cover crop biomass, it was necessary to first control for the well-documented relationship between cover crop biomass on weed suppression.

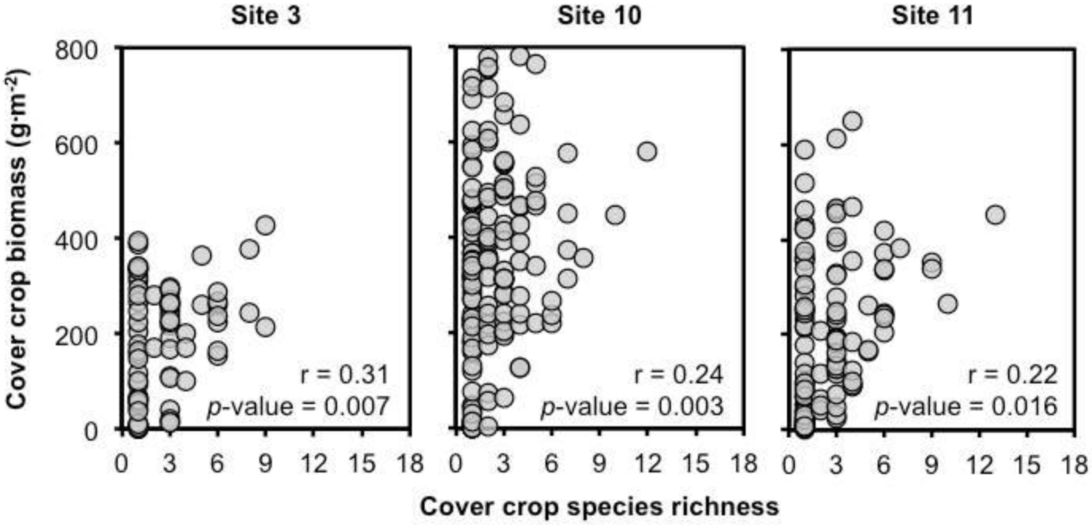

Figure 3-5. Cover crop biomass versus cover crop species richness with Pearson correlation coefficients (r) for each site. Three data points with cover crop biomass beyond 1000 g m^{-2} not shown. *P*-values are for the following hypothesis test regarding the correlation coefficients—H_0: r = 0; H_a: r ≠ 0.

Cover crop diversity and weed suppression

First, weed biomass reduction was modeled as a function of cover crop aboveground biomass (Figure 3-6). Using this as the null model, I evaluated the benefit of adding cover crop species richness as an input variable to this model. For all three sites, there was no indication that including species richness into the model improved the predictive results of the model. The parameter estimate associated with cover crop species richness, β_2, was not significantly different from zero for each site. There was only a marginal decrease (<1%) in root mean squared error (RMSE) values associated with adding the parameter β_2. Furthermore, the F-test results indicated that the information obtained from including species richness was not worth the loss in degrees of freedom (Table 3-2).

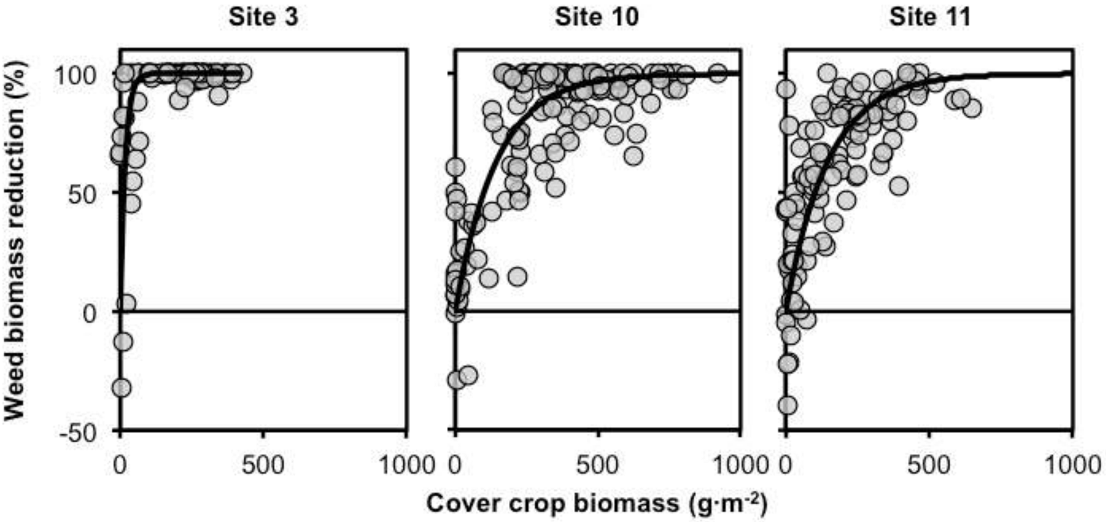

Figure 3-6. Weed biomass reduction versus cover crop biomass at each of the three sites. Exponential equation fit through each of the three data sets. Three data points with cover crop biomass beyond 1000 g m^{-2} not shown.

Table 3-2. Parameter estimates for the exponential model fitted to weed biomass reduction versus cover crop biomass for each site with and without the inclusion of cover crop species richness (SR) as a predictive variable along with F-test results.

Site	Model	df	Parameter estimates±SEM * 10^3		RMSE	F-test results	
			β_1	β_2		F-value	*p*-value
3	Null	79	57±12	-	0.205	0.49	0.49
	+ SR	78	30±18	11±11^{NS}	0.205		
10	Null	159	6.9±0.4	-	0.171	1.07	0.30
	+ SR	158	6.2±0.8	0.4±0.3^{NS}	0.170		
11	Null	119	6.8±0.5	-	0.212	0.97	0.33
	+ SR	118	7.5±0.9	-0.2±0.2^{NS}	0.211		

^{NS} Not significantly different from zero at the $\alpha = 0.05$ level.

Cover crop type and weed suppression

Grass and broadleaf cover crops had comparable effects on weed suppression regardless of whether the weeds were grasses or broadleaves (Figure 3-7).

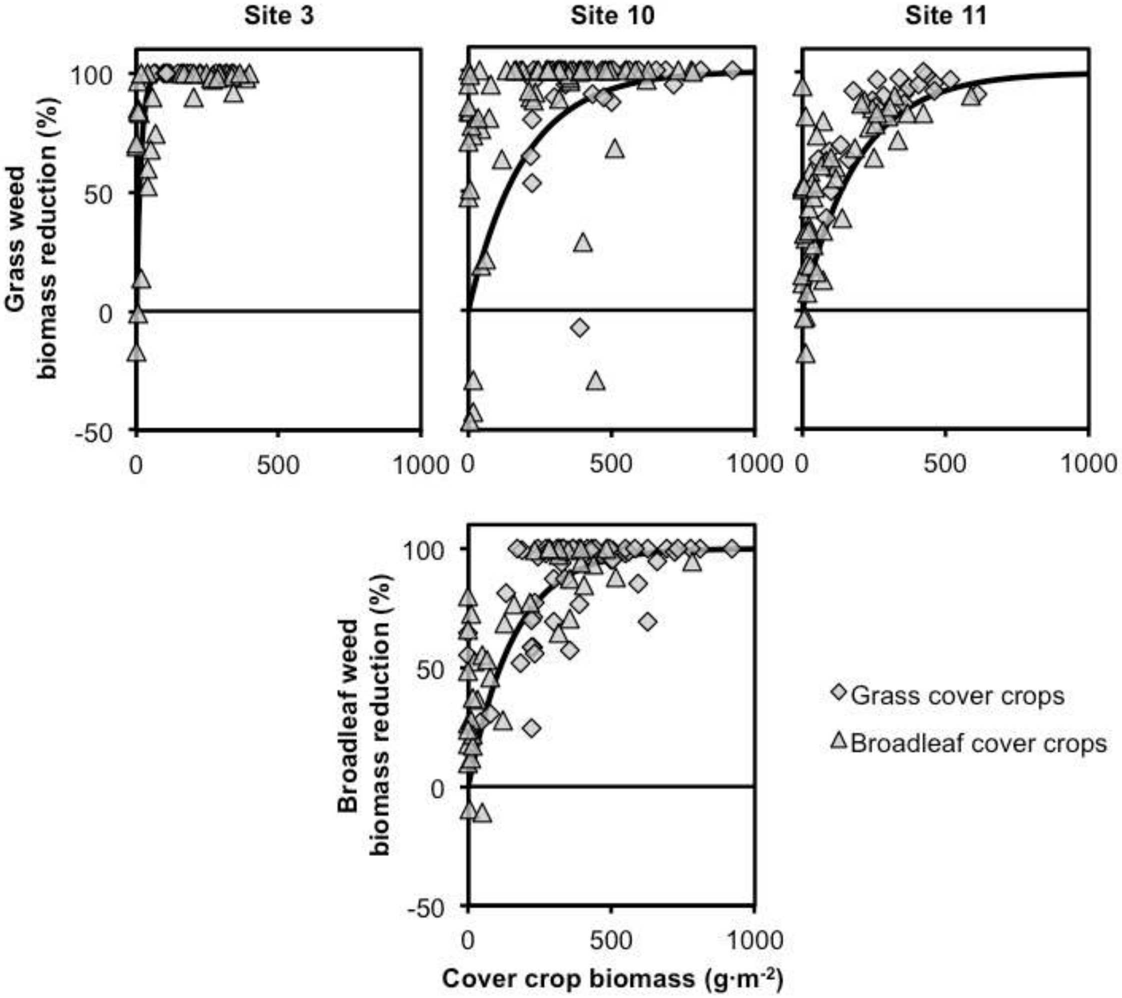

Figure 3-7. Grass and broadleaf weed biomass reduction versus cover crop biomass for grass (◇) and broadleaf (△) cover crop treatments at each of the three sites. Exponential equation fitted through each of the three data sets. Three data points with cover crop biomass beyond 1000 g m^{-2} not shown.

F-test results indicated that separation of the data on the basis of cover crop type was not worth the loss in degrees of freedom. We can also qualitatively observe that the parameter estimates for the grass and broadleaf cover crops are roughly equal at each site and for each weed type, indicating that grass and broadleaf weeds responded approximately the same to grass and broadleaf cover crops (Table 3-3; Table 3-4).

Table 3-3. Parameter estimates for the exponential model fitted to grass weed biomass reduction versus cover crop biomass for each site with grass and broadleaf cover crops separated and combined along with F-test results.

Site	Dataset	df	Parameter estimate±SEM * 10^3	F-test results	
			β_1	F-value	*p*-value
3	Grass cover crops*	15	-	-	-
	Broadleaf cover crops	35	61±19		
	Combined	51	62±16		
10	Grass cover crops	31	5±1	0.03	0.87
	Broadleaf cover crops	71	7±2		
	Combined	103	6±2		
11	Grass cover crops	23	7.6±0.6	0.52	0.47
	Broadleaf cover crops	53	6±1		
	Combined	77	6.9±0.8		

*These data points fall in a straight line around 100% weed loss at the high end of cover crop biomass productivity. Consequently, the non-linear regression methods used cannot converge on an optimum solution for the parameter β_1.

Table 3-4. Parameter estimates for the exponential model fitted to broadleaf weed biomass reduction versus cover crop biomass for site 10 with grass and broadleaf cover crops separated and combined along with F-test results.

Site	Dataset	df	Parameter estimate±SEM * 10^3	F-test results	
			β_1	F-value	*p*-value
10	Grass cover crops	31	5.3±0.4	1.77	0.19
	Broadleaf cover crops	71	7±1		
	Combined	103	6.3±0.6		

DISCUSSION

Diversity-invasibility hypothesis

Most diversity-invasibility studies are designed in such a way that biomass productivity co-varies with diversity. Most diversity-invasibility studies also then fail to control for the effect of biomass productivity on invader suppression. Consequently, what happens is that the correlation between diversity and invasion resistance is interpreted as evidence for the diversity-invasibility hypothesis, ignoring the fact that biomass productivity is driving much, if not all, of the pattern observed.

Take for example the work of Tilman (1997)—one of the more notable workers in the field of diversity relationships. In a diversity-invasibility study, he concluded, "invasibility…depended on…species richness…but was independent…of total plant cover." He came to this conclusion on the basis of a multiple regression analysis whereby species richness came out significant and total plant cover came out insignificant (Figure 3-8). Despite the conclusion being intuitively flawed—plant cover and bare soil are indisputably factors affecting invasion (e.g., Crawley, 1987; Burke and Grime, 1996)—the approach used to draw the conclusion is also statistically inappropriate. Tilman uses multiple collinear variables (e.g., species richness, total plant cover, and bare ground) in the same multiple regression to draw conclusions about causation.

While it's not certain to what degree these variables are collinear—though total plant cover and bare soil should be perfectly collinear—even low levels of collinearity can cause inaccurate model parameterization (Graham, 2003). I think it is likely that the significance of species richness and insignificance of total plant cover and bare soil in Tilman's multiple regression is spurious and a consequence of the model fitting algorithm not being able to discern how to correctly partition an effect amongst multiple collinear variables.

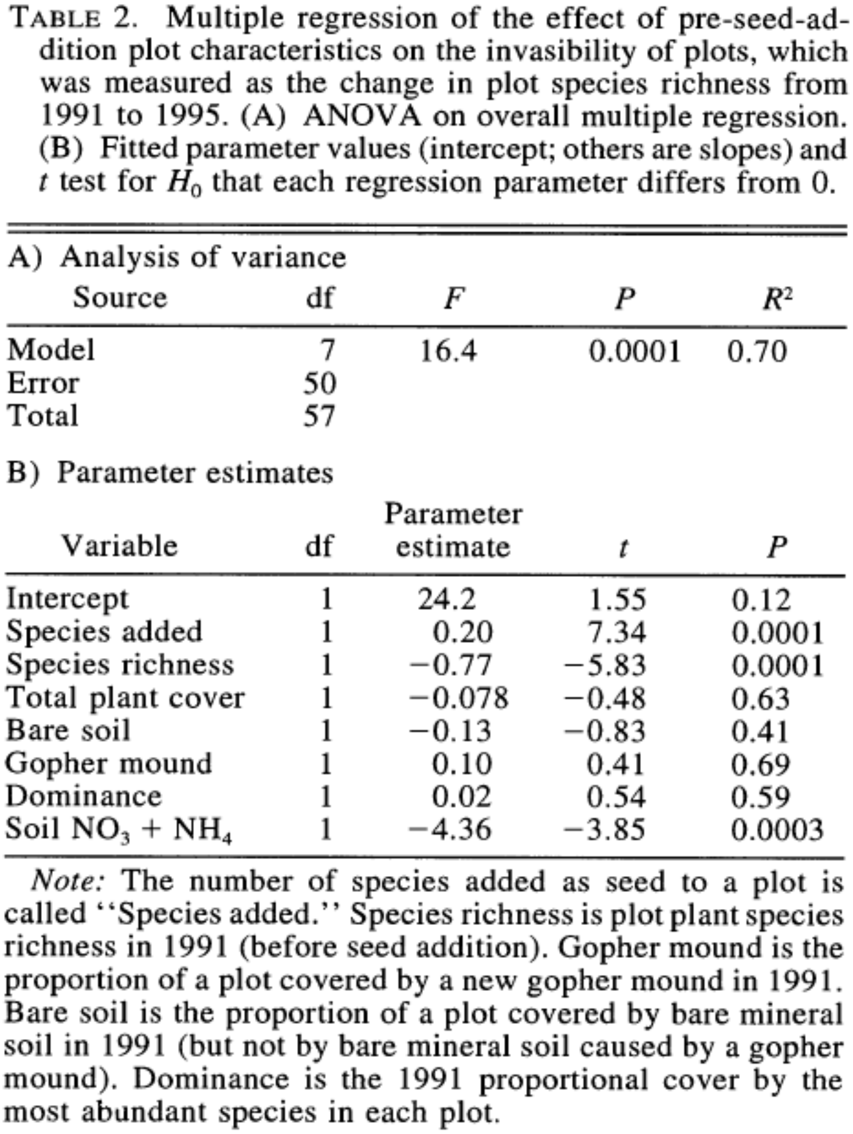

TABLE 2. Multiple regression of the effect of pre-seed-addition plot characteristics on the invasibility of plots, which was measured as the change in plot species richness from 1991 to 1995. (A) ANOVA on overall multiple regression. (B) Fitted parameter values (intercept; others are slopes) and t test for H_0 that each regression parameter differs from 0.

A) Analysis of variance

Source	df	F	P	R^2
Model	7	16.4	0.0001	0.70
Error	50			
Total	57			

B) Parameter estimates

Variable	df	Parameter estimate	t	P
Intercept	1	24.2	1.55	0.12
Species added	1	0.20	7.34	0.0001
Species richness	1	−0.77	−5.83	0.0001
Total plant cover	1	−0.078	−0.48	0.63
Bare soil	1	−0.13	−0.83	0.41
Gopher mound	1	0.10	0.41	0.69
Dominance	1	0.02	0.54	0.59
Soil $NO_3 + NH_4$	1	−4.36	−3.85	0.0003

Note: The number of species added as seed to a plot is called ''Species added.'' Species richness is plot plant species richness in 1991 (before seed addition). Gopher mound is the proportion of a plot covered by a new gopher mound in 1991. Bare soil is the proportion of a plot covered by bare mineral soil in 1991 (but not by bare mineral soil caused by a gopher mound). Dominance is the 1991 proportional cover by the most abundant species in each plot.

Figure 3-8. Reproduction of Table 2 from Tilman (1997). Results of a multiple regression analysis.

With so many studies demonstrating the negative relationship between plant cover, biomass and density with invasibility (e.g., Ateh and Doll, 1996; Barberi and Mazzoncini, 2001; Beckie et al., 2008; Blackshaw, 1993; Boerboom and Young, 1995; Brennan and Smith, 2005; Brennan et al., 2009; Chase and Mbuya, 2008; De Haan et al., 1997; Evans et al., 1991; Firbank and Watkinson, 1990; Goldberg, 1987; Hiltbrunner et al., 2007; Kristensen et al., 2008; Lawson and Topham, 1985; McLenaghen et al., 1996; Mohler and Liebman, 1987; Milbau et al., 2005; Nelson et al., 1991; Nelson et al., 2012; O'Donovan et al., 2000; O'Donovan, 1994; Ross et al., 2001; Ryan et al., 2011; Uchino et al., 2012; VanderVorst et al., 1983; Weiner et al., 2001; Wicks et al., 2004), it makes little sense that total plant cover and the amount of bare ground have no effect on invasiblility while species richness does. This highlights one of the major flaws of using multiple regression to determine the effect of diversity on invasibility

In a similar vein, agronomic experiments that seek to show the increased weed suppression of plant mixtures often fail to take into account the increased biomass of plant mixtures in many experiments. Once we take into account the effect of plant mixture or crop productivity on weed suppression, the apparent effect of diversity often falls away. Take for example a study by Szumigalski and Van Acker (2005) on the effects of mixing wheat, canola, and field pea on weed suppression. The authors conclude, "annual intercrops can enhance…weed suppression…compared with sole crops." However, once we account for the effect of crop biomass on weed biomass, we find that crop diversity doesn't explain any additional variation in weed suppression (Figure 3-9). There is no evidence that plant mixtures "enhance" weed suppression compared with plant monocultures.

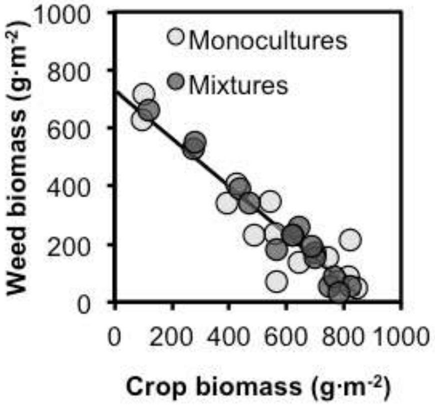

Figure 3-9. Mean dry weed biomass versus mean dry crop biomass for wheat, pea, and canola in monoculture and mixtures. Data is from Szumigalski and Van Acker (2005). Data is aggregated across two sites—Kelburn and Carmen, Manitoba—and two years—2002 and 2003. Data from 2001 is omitted due to low weed pressure. Linear regression: $y = 729.1 - 0.8x$.

Review papers of mixed cropping literature regularly give the impression that it's the actual mixing of crops that is promoting weed suppression (Liebman and Davis, 1999; Liebman and Dyck, 1993; Masiunas, 1998) without addressing the possibility that it could simply be increased biomass increasing weed suppression. However, what would all those studies look like if we took into account variations in crop biomass production as we did with the Szumigalski and Van Acker (2005) data? Would we find that it is not so much intercrops that are superior at suppressing weeds but rather productive crops?

Furthermore, if we use the increased weed suppressiveness of intercrops as evidence of increased resource use efficiency of intercrops, what do we do with cases where the sole crops are more suppressive than the intercrops (e.g., Arny et al., 1929;

Mohler and Liebman, 1987)? Do we then take those results and say that sole crops are more nutrient use efficient than intercrops? Liebman (1995) explains this inconsistency by asserting that the hypothesis that diverse systems are better at pre-empting resource use is perhaps just true in some instances but not others. I think this is a weak assertion and assert that to explain this seeming inconsistency, we need to look no further than to variations in biomass (Gomez and Gurevitch, 1998; Nelson et al., 2012). Returning to the example of Mohler and Liebman (1987), the sole crops that were more suppressive of weeds than the intercrops were also more productive than the intercrops (Figure 3-10).

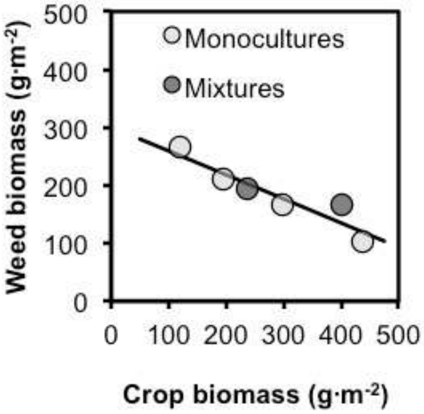

Figure 3-10. Mean dry weed biomass versus mean dry crop biomass for barley and pea both in monoculture and mixtures. Data from Mohler and Liebman (1987) from Turkey Farm. Linear regression: y = 302.0 – 0.4x.

Despite these issues in data analysis and results interpretation, however, studies which confound the effects of diversity and productivity continue to proliferate and to conclude a negative relationship between diversity and invasibility (Biondini, 2007;

Dukes, 2001; Dukes, 2002; Fargione and Tilman, 2005; Fargione et al., 2003; Jiang et al., 2007; Kennedy et al., 2002; Knops et al., 1999; Levine, 2000; Naeem et al., 2000; Picasso et al., 2008; Pfisterer et al., 2004; Prieur-Richard et al., 2000; Symstad, 2000; van Ruijven et al., 2003; Zavaleta and Hulvey, 2004; Zavaleta and Hulvey, 2007). This issue includes subsequent meta-analyses consolidating these findings (Balvanera et al., 2006; Levine et al., 2004). And through sheer re-iteration, this highly questionable hypothesis has developed the patina of ecological principle.

While our study doesn't disprove the diversity-invasibility hypothesis, it highlights one of the major issues underlying most of the supposed evidence for diversity-invasibility hypothesis—the covariance of diversity with productivity. Goldberg and Werner (1983) made an early call for scientists to account for the effects of biomass when studying plant invasion, but overwhelmingly their advice has been ignored with regard to the study of the effect of diversity on invasibility. After accounting for the well-documented effect of plant productivity on weed suppression in this study, there was no observable effect of cover crop species richness on invasibility. This is consistent with the findings of Lanta and Lepš (2008) who also controlled for the effect of resident biomass on invader biomass prior to testing for the effect of species and functional richness.

Competition-relatedness hypothesis

With regard to competition-relatedness, we found no evidence that grasses were more suppressive of grasses or that broadleaves were more suppressive of broadleaves. Weed suppression was largely a function of cover crop productivity rather than cover crop type. Take for example, data from Nelson et al. (1991) on the weed suppression of

14 different grass and legume cover crops (Figure 3-11). Most of the variation in weed suppression can be explained by cover crop ground coverage. Once variation in ground cover is accounted for, there is little difference in the weed suppression of grasses versus legumes.

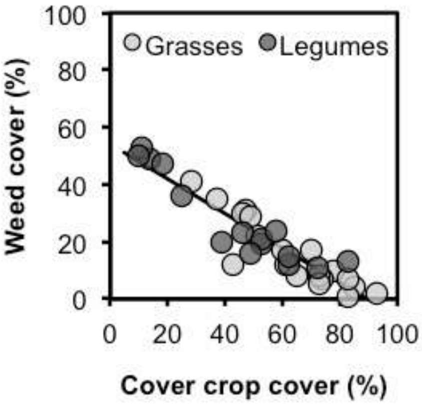

Figure 3-11. Mean percent weed cover versus mean percent cover crop cover for fourteen grass and legume cover crops. Data from Nelson et al. (1991). Data from two sites—Bixby and Lane, Oklahoma—and two years—1987 and 1988. Linear regression: y = 53.9 - 0.6x.

The results of this study force us to think concretely about the logical underpinnings of the competition-relatedness hypothesis. That is, why would we think a grass cover crop would be better at suppressing a grass weed than a broadleaf cover crop? It's easy to be glib and say that two grass species would occupy a more similar niche than a grass and a broadleaf, and thus a grass cover crop would be better at displacing a grass weed, but what does that really mean when we try to unpack it?

Parting thoughts regarding diversity and resource pre-emption

Empirical evidence aside, I think there are important logical arguments to be made against both the diversity-invasibility hypothesis and the competition relatedness-hypothesis. Let’s start with the competition-relatedness hypothesis, which is in many ways a complement to the diversity-productivity hypothesis discussed in Chapter 2. One of the ideas underpinning the diversity-productivity hypothesis is that a single species has a particular niche in an environment and a monoculture of that single species leaves other niches in that environment unexploited, which another species could come in and use—this ties in with the idea of resource use efficiency. The competition-relatedness hypothesis is simply a variation of the diversity productivity hypothesis. Rather than think about increasing productivity by filling vacant niches with new species, however, it considers how to repel invaders by filling vacant niches. It presumes that a like species will be most able to repel a like invader because their needs will be so similar, and consequently a like species will be best able to pre-empt all the resources needed by an invader.

What seems so remarkable to me is how entrenched this argument is despite the lack of empirical evidence in favor of it (Cahill et al., 2008) and how flimsy it is with regards to the simplest of examples. Let’s take the example of red clover and yellow blossom sweet clover—two very similar species—and turnip—a very unlike species. The competition-relatedness hypothesis predicts that competition will be more severe between the two clovers than either of the clovers and the turnip on the basis of their occupying similar niches. For anyone who has seen these three species growing, it’s clear that turnip is much more competitive with the clovers than they are with each other. It grows aggressively and shades out the clovers rapidly, whereas the clovers can co-exist easily. It doesn’t matter that the two clovers have more similar growth habits and nutrient needs. It matters that the turnip can better pre-empt the single limiting resource, light. The competitor doesn’t need to pre-empt the use of every resource, just one. Being an effective competitor is more about capturing a key resource than it is about occupying a similar niche to the species being competed with. This is key to not only unhinging the competition-relatedness hypothesis, but also dismantling the diversity-invasibility hypothesis.

The logic of the diversity-invasibility hypothesis goes like this:

1. The key to preventing invaders is to pre-empt the use of the resources in an environment.
2. A diverse community is better able to fully use the finite resources in an environment than a less diverse community
3. Therefore, a diverse system is more resistant to invasion because it more fully uses available resources.

As Liebman and Staver (2004) put it with regard to crop diversity:“[b]ecause annual crop mixtures often exploit a greater range and quantity of resources than sole crops, they can be more effective for suppressing weeds through resource preemption.” While there’s a sort of intuitive elegance to this at first glance, I think the more we scrutinize these assumptions, the more the diversity-invasibility hypothesis unravels.

It is neither feasible nor necessary to fully use all the available plant resources to suppress weeds. Imagine what it would mean to fully use all the nutrients in the soil, all the water in the soil, and all the carbon dioxide and oxygen in the air to pre-empt their use by weeds. It’s not feasible. However, what is feasible is using all the incoming solar radiation. Light impedance without any interference with any other resource is sufficient for complete weed suppression. This is how mulches and shade cloths can be so successful at weed suppression (Teasdale, 1993). Furthermore, a densely planted monoculture can be quite efficient at intercepting incoming solar radiation (e.g., Teasdale, 1995; Norsworthy and Oliveira, 2004; Tharp and Kells, 2001). A mixture is not required for maximizing light interception. It should be noted that while in this study I have focused on cover crop biomass as the explanatory variable, in truth light transmittance is likely the more ultimate explanatory variable with biomass simply being an imperfect but functional proxy measurement. Both light transmittance and weed suppression demonstrate exponential decay patterns with increasing biomass (Teasdale, 1997; Teasdale and Mohler, 1993).

Not only is empirical evidence for the diversity-invasibility and competition-relatedness hypothesis lacking with regards to plant mixtures, I find the logical foundation on which these hypotheses stands suspect. In Chapter 4, I continue to explore these ideas relating diversity to resource use efficiency by evaluating the relationship between cover crop mixture diversity and soil nutrient capture.

References

Altieri, M.A., and M. Liebman. 1986. Insect, weed, and plant disease management in multiple cropping systems. In: C.A. Francis, editor, Multiple Cropping Systems. Macmillan, New York, NY. p. 183-218.

Anil, L., J. Park, R. H. Phipps, and F. A. Miller. 1998. Temperate intercropping of cereals for forage: A review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53:301-317.

Arny, A. C., T. E. Stoa, C. McKee, and A. C. Dillman. 1929. Flax cropping in mixture with wheat, oats, and barley. Tech. Bull. 133. USDA: Washington, D. C.

Ateh, C. M., and J. D. Doll. 1996. Spring-planted winter rye (*Secale cereale*) as a living mulch to control weeds in soybean (*Glycine max*). Weed Technol. 10:347-353.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. 9(10):1146-1156.

Barberi, P., and M. Mazzoncini. 2001. Changes in weed community composition as influenced by cover crop and management system in continuous corn. Weed Sci. 49:491-499.

Beckie, H. J., E. N. Johnson, R. E. Blackshow, and Y. Gan. 2008. Weed Suppression by Canola and Mustard Cultivars. Weed Technol. 22(1):182-185.

Biondini, M. 2007. Plant diversity, production, stability, and susceptibility to invasion in restored northern tall grass prairies (United States). Restor. Ecol. 15:77-87.

Blackshaw, R. E. 1993. Safflower (*Carthamus tinctorius*) Density and Row Spacing Effects on Competition with Green Foxtail (*Setaria viridis*). Weed Sci. 41:403-408.

Boerboom, C. M., and F. L. Young. 1995. Effect of Postplant Tillage and Crop Density on Broadleaf Weed Control in Dry Pea (*Pisum satibum*) and Lentil (*Lens culinaris*). Weed Technol. 9(1):99-106.

Brennan, E. B., and R. F. Smith. 2005. Winter Cover Crop Growth and Weed Suppression on the Central Coast of California. Weed Technol. 19:1017-1024.

Brennan, E. B., N. S. Boyd, R. F. Smith, and P. Foster. 2009. Seeding Rate and Planting Arrangement Effects on Growth and Weed Suppression of a Legume-Oat Cover Crop for Organic Vegetable Systems. Agron. J. 101(4): 979-988.

Buhler, D. D. 2003. Weed Biology, Cropping Systems, and Weed Management. In: A. Shrestha, editor, Cropping Systems: Trends and Advances. Haworth Press, Binghamton, NY. p. 245-270.

Burke, M. J. W., and J. P. Grime. 1996. An Experimental Study of Plant Community Invasibility. Ecology 77(3):776-790.

Cahill, J. F., Jr., S. W. Kembel, E. G. Lamb, and P. A. Keddy. 2008. Does phylogenetic relatedness influence the strength of competition among vascular plants. Perspect. Plant Ecol. 10:41-50.

Chase, C. A., and O. S. Mbuya. 2008. Greater Interference from Living Mulches than Weeds in Organic Broccoli Production. Weed Technol. 22(2):280-285.

Crawley, M. J. 1987. What makes a community invasible? In: A. J. Gray, M. J. Crawley, and P. J. Edwards, editors, Colonization, succession, and stability. Blackwell, Oxford, UK. p. 429-453.

Darwin, C. 1872. On the origin of species by means of natural selection. Murray, London.

De Haan, R., C. C. Sheaffer, and D. K. Barnes. 1997. Effect of Annual Medic Smother Plants on Weed Control and Yield. Agron. J. 89:813-321.

Dukes, J. S. 2001. Biodiversity and Invasibility in Grassland Microcosms. Oecologia 126(4):563-568.

Dukes, J. S. 2002. Species composition and diversity affect grassland susceptibility to invasion. Ecol. Appl. 12(2):602-617.

Elton, C.S. 1958. The ecology of invasions by plants and animals. Chapman and Hall, London, UK.

Evans, T. M., D. C. Thill, L. Tapia, B. Shafii, and J. M. Lish. 1991. Wild Oat (*Avena fatua*) and Spring Barley (*Hordeum vulgare*) Density Affect Spring Barley Grain Yield. Weed Technol. 5(1):33-39.

Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. Ecol. Lett. 8:604-611.

Fargione, J. E., C. S. Brown, and D. Tilman. 2003. Community assembly and invasion: An experimental test of neutral versus niche processes. PNAS 100(15):8916-8920.

Firbank, L. G., and A. R. Watkinson. 1990. On the effects of plant competition: from monocultures to mixtures. In: J. Grace and D. Tilman, editors, Perspectives on Plant Competition. Academic Press, New York, NY. p. 165-192.

Goldberg, D. E. 1987. Neighborhood Competition in an Old-Field Plant Community. Ecology 68(5):1211-1223.

Goldberg, D. E., and P. A. Werner. 1983. Equivalence of competitors in plant communities: a null hypothesis and a field experimental approach. Am. J. Bot. 70(7):1098-1104.

Gomez, P., and J. Gurevitch. 1998. Weed community responses in a corn-soybean intercrop. Appl. Veg. Sci. 1:281-288.

Graham, M. H. 2003. Confronting multicollinearity in ecological multiple regression Ecology 84(11):2809-2815.

Grothendieck, G. 2013. nls2: Non-linear regression with brute force. R package version 0.2. http://CRAN.R-project.org/package=nls2

Hiltbrunner, J., M. Liedgens, L. Bloch, P. Stamp, and B. Streit. 2007. Legume cover crops as living mulches for winter wheat: Components of biomass and the control of weeds. Eur. J. Agron. 26:21-29.

Huston, M.A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110:449-460.

Jiang, X. L., W. G. Zhang, and G. Wang. 2007. Biodiversity effects on biomass production and invasion resistance in annual versus perennial plant communities. Biodivers. Conserv. 16:1983-1994.

Kennedy, T. A., S. Naeem, K. M. Howe, J. M. H. Knops, D. Tilman, and P. Reich. 2002. Biodiversity as a barrier to ecological invasion. Nature 417(6):636-638.

Knops, J. M. H., D. Tilman, N. M. Haddad, S. Naeem, C. E. Mitchell, J. Haarstad, M. E. Ritchie, K. M. Howe, P. B. Reich, E. Siemann, and J. Groth. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecol. Lett. 2:286-293.

Kristensen, L., J. Olsen, and J. Weiner. 2008. Crop Density, Sowing Pattern, and Nitrogen Fertilization Effects on Weed Suppression and yield in Spring Wheat. Weed Sci. 56(1):97-102.

Lanta, V., and J. Lepš. 2008. Effect of Plant Species Richness on Invasibility of Experimental Plant Communities. Plant Ecol. 198(2):253-263.

Lawson, H. M., and P. B. Topham. 1985. Competition between annual weeds and vining peas grown at a range of population densities: effects on the weeds. Weed Res. 24:221-229.

Levine, J. M, and C. M. D'Antonio. 1999. Elton revisited: a review of evidence linking diversity and invasibility. Oikos 87:15-26.

Levine, J. M. 2000. Species Diversity and Biological Invasions: Relating Local Process to Community Pattern. Science 288(5467):852-854.

Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. Ecol. Lett. 7:975-989.

Liebman, M, and C. P. Staver. 2004. Crop diversification for weed management. In: M. Liebman, C. L. Mohler, and C. P. Staver, editors, Ecological Management of Agricultural Weeds. Cambridge University Press, Cambridge, UK. p. 322-374.

Liebman, M. 1995. Polyculture cropping systems. In: M. Altieri, editor, Agroecology: The science of sustainable agriculture. 2nd ed. Westview Press, Boulder, CO. p. 205-218.

Liebman, M., and A. S. Davis. 1999. Integration of soil, crop and weed management in low-external-input farming systems. Weed Res. 40:27-47.

Liebman, M., and E. Dyck. 1993. Crop Rotation and Intercropping Strategies for Weed Management. Ecol. Appl. 3(1):92-122.

Masiunas, J. B. 1998. Production of Vegetables Using Cover Crop and Living Mulches—A Review. J. Vegetable Crop Production 4(1):11-31.

McLenaghen, R. D., K. C. Cameron, N. H. Lampkin, M. L. Daley, and B. Deo. 1996. Nitrate leaching from ploughed pasture and the effectiveness of winter catch crops in reducing leaching losses. New Zeal. J. Agr. Res. 39(3):413-420.

Milbau, A., I. Nijs, F. De Raedemaecker, D. Reheul, and B. De Cauwer. 2005. Invasion in Grassland Gaps: The Role of Neighborhood Richness, Light Availability and Species Complementarity during Two Successive Years. Funct. Ecol. 19(1):27-37.

Mohler, C. L., and M. Liebman. 1987. Weed productivity and composition in sole crops and intercrops of barley and field pea. J. Appl. Ecol. 24:685-699.

Moody, K. 1977. Weed control in multiple cropping. In: Proceedings of the Symposium on Cropping Systems Research and Development for the Asian Riche Farmer. 21-27 Sept. 1976. IRRI, Los Baños, Phillipines. p. 281-294

Moody, K., and S. V. R. Shetty. 1981. Weed Management in Intercropping Systems. In: R. W. Willey, editor, Proceedings of the International Workshop on Intercropping. 10-13 Jan. 1979. ICRISAT, Hyderabad, India. p. 229-237.

Motulsky, H., and A. Christopoulos. 2003. Fitting models to biological data using linear and nonlinear regression:a practical guide to curve fitting. GraphPad Software Inc., San Diego, CA. www.graphpad.com

Naeem, S., J. M. H. Knops, D. Tilman, K. M. Howe, T. Kennedy, and S. Gale. 2000. Plant Diversity Increases Resistance to Invasion in the Absence of Covarying Extrinsic Factors. Oikos 91(1):97-108.

Nelson, A. G., A. Pswarayi, S. Quideau, B. Frick, and D. Spaner. 2012. Yield and Weed Suppression of Crop Mixtures in Organic and Conventional Systems of the Western Canadian Prairie. Agron. J. 104:756-762.

Nelson, W. A., B. A. Kahn, amd B. W. Roberts. 1991. Screening cover crops for use in conservation tillage following spring plowing. HortScience 26:860-862.

Norsworthy, J. K., and M. J. Oliveira. 2004. Comparison of the Critical Period for Weed Control in Wide and Narrow Row Corn. Weed Sci. 52(5):802-807.

O'Donovan, J. T. 1994. Canola (*Brassica rapa*) Plant Density Influences Tartary Buckwheat (*Fagopyrum tataricum*) Interference, Biomass, and Seed Yield. Weed Sci. 42(3):385-389.

O'Donovan, J. T., K. Neil Harker, G. W. Clayton, and L. M. Hall. 2000. Wild Oat (*Avena fatua*) Interference in Barley (*Hordeum vulgare*) Is Influenced by Barley Variety and Seeding Rate. Weed Technol. 14(3):624-629.

Pfisterer, A. B., J. Joshi, B. Schmid, and M. Fischer. 2004. Rapid decay of diversity-productivity relationships after invasion of experimental plant communities. Basic Appl. Ecol. 5:5–14.

Picasso, V. D., E. C. Brummer, M. Liebman, P. M. Dixon, and B. J. Wilsey. 2008. Crop Species Diversity Affects Productivity and Weed Suppression in Perennial Polycultures under Two Management Strategies. Crop Sci. 48:331-342.

Prieur-Richard, A.-H., S. Lavorel, K. Grigulis, and A. Dos Santos. 2000. Plant community diversity and invisibility by exotics: the example of *Conyza bonariensis* and *Conyza canadensis* invasion in Mediterranean annual old fields. Ecol. Lett. 3:412-422.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Richardson, D. M., and P Pyšek. 2006. Plant invasions: merging the concepts of species invasiveness and community invasibility. Prog. Phys. Geog. 30(3):409-431.

Ross, S. M., J. R. King, R. C. Izaurralde, and J. T. O'Donovan. 2001. Weed Suppression by Seven Clover Species. Agron. J. 93:820-827.

Ryan, M. R., W. S. Curran, A. M. Grantham, L. K. Hunsberger, S. B. Mirskey, D. A. Mortensen, E. A. Nord, and D. O. Wilson. 2011. Effects of Seeding Rate and Poultry Litter on Weed Suppression from a Rolled Cereal Rye Cover Crop. Weed Sci. 59(3):438-444.

Smith, R. G., L. W. Atwood, and N. D. Warren. 2014. Increased Productivity of a Cover Crop Mixture is Not Associated with Enhanced Agroecosystem Services. PLoS ONE 9(5): e97351. doi:10.1371/journal.pone.0097351

Symstad, A. J. 2000. A Test of the Effects of Functional Group Richness and Composition on Grassland Invasibility. Ecology 81(1):99-109.

Szumigalski, A., and R. Van Acker. 2005. Weed Suppression and Crop Production in Annual Intercrops. Weed Sci. 53(6):813-825.

Teasdale, J. R. 1993. Interaction of light, soil moisture, and temperature with weed suppression by hairy vetch residue. Weed Sci. 41:46-51.

Teasdale, J. R. 1995. Influence of Narrow Row/High Population Corn (*Zea mays*) on Weed Control and Light Transmittance. Weed Technol. 9(1):113-118.

Teasdale, J. R. 1998. Cover crops, smother plants, and weed management. In: J. L. Hatfield, D.D. Buhler, and B.A. Stewart, editors, Integrated weed and soil management. Ann Arbor Press, Chelsea, MI. p. 247-270

Teasdale, J. R., and C. L. Mohler. 1993. Light transmittances, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron. J. 85: 673-680.

Teasdale, J. R., L. O. Brandsæter, A. Calegari, and F. Skora Neto. 2007. Cover Crops and Weed Management. In: M. K. Upadhyaya and R. E. Blackshaw, editors, Non-chemical Weed Management. CAB International, Cambridge, MA. p. 49-64.

Tharp, B. E. and J. J. Kells. 2001. Effect of glufosinate-resistantcorn (*Zea mays*) population and row spacing on light interception, corn yield, and common lambsquarters (*Chenopodium album*) growth. Weed Technol. 15:413-418.

Tilman, D. 1997. Community Invasibility, Recruitment Limitation, and Grassland Biodiversity. Ecology 78(1):81-92.

Tracy, B. F., I. J. Renne, J. Gerrish, and M. A. Sanderson. 2004. Effects of plant diversity on invasion of weed species in experimental pasture communities. Basic Appl. Ecol. 5:543-550.

Uchino, H., K. Iwama, Y. Jitsuyama, K. Ichiyama, E. Sugiura, T. Yudate, S. Nakamura, and J. Gopal. 2012. Effect of interseeding cover crops and fertilization on weed suppression under an organic and rotational cropping system 1. Stability of weed suppression over years and main crops of potato, maize and soybean. Field Crop. Res. 127:9-16.

van Ruijven, J., G. B. De Deyn, and F. Berendse. 2003. Diversity reduces invasibility in experimental plant communities: the role of plant species. Ecol. Lett. 6:910-918.

Vander Vorst, P. B., G. A. Wicks, and O. C. Burnside. 1983. Weed control in a winter wheat-corn-ecofarming rotation. Agron. J. 75:507-511.

Wardle, D. A. 2001. Experimental Demonstration That Plant Diversity Reduces Invasibility: Evidence of a Biological Mechanism or a Consequence of Sampling Effect? Oikos 95(1):161-170.

Weiner, J., H.-W. Griepentrop, and L. Kristensen. 2001. Suppression of Weeds by Spring Wheat *Triticum aestivum* increases with crop density and spatial uniformity. J. Appl. Ecol. 38:784-790.

Wicks, G. A., P. T. Nordquist, P S. Baenziger, R. N. Klein, R. H. Hammons, and J. E. Watkins. 2004. Winter Wheat Cultivar Characteristics Affect Annual Weed Suppression. Weed Technol. 18(4):988-998.

Zavaleta, E.S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland resistance to biological invaders. P. Natl. A. Sci. USA 306:1175–1177.

Zavaleta, E.S., and K. B. Hulvey. 2007. Realistic variation in species composition affects grassland production, resource use and invasion resistance. Plant Ecol. 188:39-51.

Chapter 4 - Cover crop mixture diversity and soil nutrient retention

ABSTRACT

The purpose of this study was to test the hypothesis that increasing plant mixture diversity increases soil nutrient retention. Specifically, this study evaluated the effect of increasing cover crop mixture species richness on soil extractable nitrogen (NO_3-N), phosphorus (P), potassium (K), chloride (Cl), and sulfur (SO_4-S) concentrations and distributions. Twenty treatments composed of one no cover control treatment, nine monoculture treatments, and ten mixture treatments reflecting varying levels of species richness were replicated four times in a field at Hooper, Nebraska. Cover crops were planted after wheat harvest on August 31, 2013. Plant aboveground biomass samples were taken October 31, 2013. Soil samples were taken from 0-10 cm in all plots and additionally from 10-20, 20-30, and 30-60 cm in a select subset of treatments on April 9, 2014. Cover cropping increased the concentration of the relatively mobile nutrients—NO_3-N, SO_4-S, and Cl —in the top 10 cm, and generally decreased their concentration in the lower depths—suggesting decreased leaching of these nutrients under cover cropping. K concentrations were increased throughout the soil profile under cover cropping—suggesting perhaps increased weathering of K bearing minerals in addition to possible decreased leaching under cover cropping. P concentrations were not consistently affected by cover cropping. Where cover cropping affected soil nutrient retention, many of these effects were further mediated by plant biomass. Controlling for the relationship between plant biomass and soil nutrient retention, there was no evidence that increasing cover crop species richness increased soil nutrient retention or that cover crop mixtures retained more soil nutrients than cover crop monocultures.

INTRODUCTION

It's well documented that cover crops can be used to decrease soil nutrient leaching losses—particularly of those nutrients that are relatively mobile in the soil. However, it's not clear to what extent cover crop mixture diversity affects the ability of a cover crop to retain soil nutrients. Different plant species certainly have different root and shoot architectures that develop at different rates over different time frames. However, does this spatial and temporal diversity in root and shoot development translate into improved soil nutrient retention? It has been hypothesized that increasing plant mixture diversity should decrease nutrient leaching losses and increase nutrient retention—the diversity-nutrient retention hypothesis—but empirical evidence is limited (Tilman et al., 1996; Tilman et al., 2001; Vitousek and Hooper, 1994). The objective of this study was to test this hypothesis in the context of cover crop mixtures and soil nutrient retention. Specifically, this study evaluated the effect of cover crop mixture species richness on soil extractable nitrate, phosphorus, potassium, sulfate, and chloride concentrations and distributions.

Of all soil nutrients, soil nitrate has received the most attention with regards to cover crops. The ability of cover crops to decrease nitrate leaching has been well documented (e.g., Askegaard et al., 2011; Brandi-Dohrn et al., 1997; Martinez and Guiraud, 1990; Isse et al., 1999; rev. Kirchmann et al., 2002; Lewan, 1994; McLenaghen et al., 1996; rev. Meisinger et al., 1991; Milburn et al., 1997; Poudel et al., 2001; Shepherd, 1999; Shepherd and Lord, 1996; Sørensen, 1991; Strock et al., 2004; Thomsen, 2005; Weinert et al., 2002; Wyland et al., 1996). Like nitrate, sulfate and chloride are also highly mobile in the soil (Bray, 1954). Predictably then, their loss from the soil is also diminished by the presence of standing vegetation (Erikson and Thorup-Kristensen, 2002; Kauffman et al., 2003; Allison et al., 1959).

Phosphorus and potassium are relatively immobile as compared to nitrate, sulfate, and chloride, and consequently little work has been conducted on the effect of vegetation on their retention in the soil. However, while these nutrients are relatively immobile, they are not completely immobile. Soil potassium leaching losses under cropland conditions have been documented to range from 0 to 245 kg ha^{-1} yr^{-1} with cropped soils tending to have less potassium leaching losses than uncropped soils (Allison et al., 1959; Bertsch and Thomas, 1985; Nolan and Pritchett, 1960; Quémener, 1986; Truog and Jones, 1938). Soil phosphorus leaching losses are typically less than soil potassium leaching losses and have been documented to range from 0.03 to 1.85 kg ha^{-1} yr^{-1} with minimal effect of cropping observed on soil phosphorus losses (Allison et al., 1959; Culley et al., 1983; Djodjic et al., 2004; Turtola and Jaakola, 1995).

While the effect of vegetation on soil nutrient retention has been relatively well studied, it's not clear to what extent plant mixture diversity affects soil nutrient retention. In a meta-analysis, Balvanera et al. (2006) estimated there to be a positive effect of plant diversity on soil nutrient supply. Unfortunately, the authors' link to the data they used for their meta-analysis is defunct, so it is difficult to see the literature they used to draw this conclusion. In my own survey of the literature, however, I find the evidence to be much less conclusive.

In a constructed grassland experiment, Tilman et al. (1996) found that increasing species richness was associated with decreased amounts of soil nitrate. They interpreted this to indicate that richer communities are able to take up greater amounts of soil nitrate.

However, it's important to note that plant species richness in this experiment was correlated with plant productivity as estimated by total plant cover. Thus, the effects of increased plant diversity on soil nutrient retention are confounded with the effects of increased plant productivity on soil nutrient retention. Furthermore, Tilman and his colleagues again misuse and misinterpret multiple regression to come to their conclusions (see Chapter 3).

Tilman et al. regressed soil nitrate against species richness, plant total cover, and root mass in a multiple regression. They took the significance of the parameter estimate associated with species richness and the insignificance of the parameter estimate associated with plant total cover and root mass to conclude that "soil NO_3^- … was independent of plant cover and surface root biomass but…dependent on species richness" (Figure 4-1).

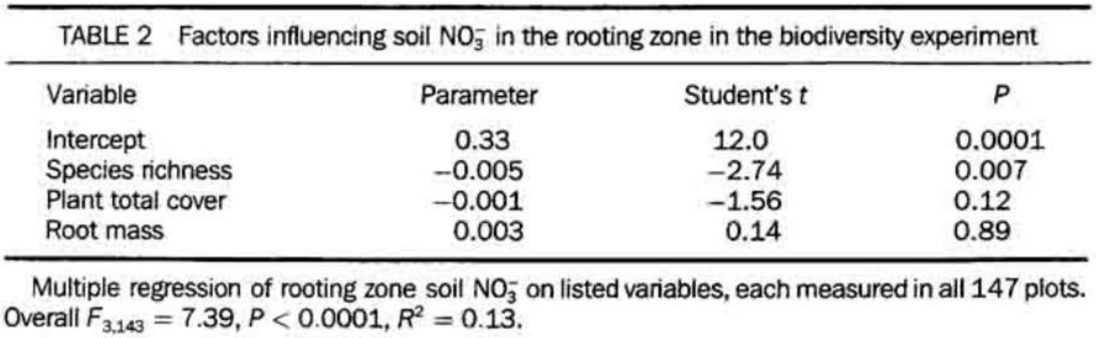

TABLE 2 Factors influencing soil NO_3^- in the rooting zone in the biodiversity experiment

Variable	Parameter	Student's *t*	*P*
Intercept	0.33	12.0	0.0001
Species richness	−0.005	−2.74	0.007
Plant total cover	−0.001	−1.56	0.12
Root mass	0.003	0.14	0.89

Multiple regression of rooting zone soil NO_3^- on listed variables, each measured in all 147 plots. Overall $F_{3,143} = 7.39$, $P < 0.0001$, $R^2 = 0.13$.

Figure 4-1. Reproduction of Table 2 from Tilman et al. (1996). Results of a multiple regression analysis.

This is an inappropriate use of multiple regression. Species richness, total plant cover, and root mass co-vary in this biodiversity experiment (Mueller et al., 2013; Tilman et al., 1996). Consequently, the effects of these three variables on soil nutrient retention are confounded. The issues with using multiple correlated variables in a multiple regression are discussed in Chapter 3, but generally speaking, feeding multiple correlated variables into a multiple regression can lead to inaccurate model parameterization because the model fitting procedure is unable to discern how to correctly partition variability between correlated variables (Graham, 2003).

This inappropriate use of multiple regression becomes xmore apparent when Tilman et al. regress plant productivity against soil nitrate, observe a significant, negative parameter estimate on soil nitrate and conclude that “total plant cover in the diversity experiment was negatively dependent on rooting zone soil NO_3^- ” (Figure 4-2).

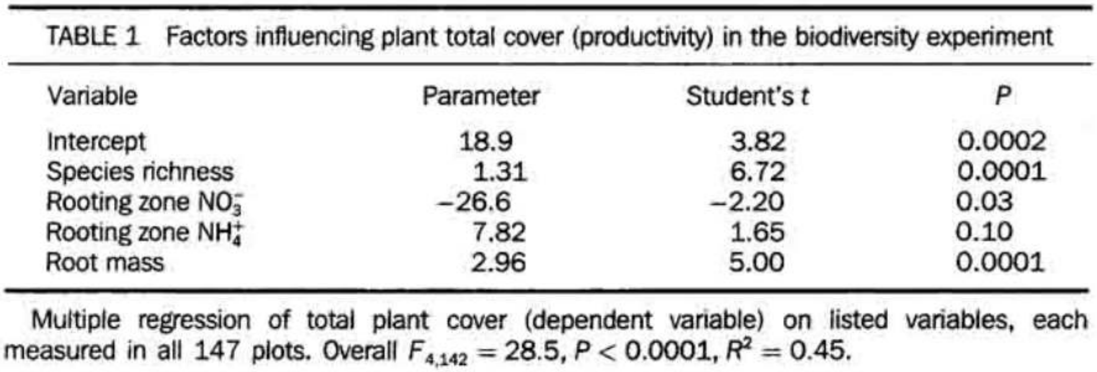

TABLE 1 Factors influencing plant total cover (productivity) in the biodiversity experiment

Variable	Parameter	Student's *t*	*P*
Intercept	18.9	3.82	0.0002
Species richness	1.31	6.72	0.0001
Rooting zone NO_3^-	−26.6	−2.20	0.03
Rooting zone NH_4^+	7.82	1.65	0.10
Root mass	2.96	5.00	0.0001

Multiple regression of total plant cover (dependent variable) on listed variables, each measured in all 147 plots. Overall $F_{4,142} = 28.5$, $P < 0.0001$, $R^2 = 0.45$.

Figure 4-2. Reproduction of Table 1 from Tilman et al. (1996). Results of a multiple regression analysis.

Thus, Tilman et al. conclude both that plant cover is unrelated to soil nitrate (Figure 4-1) and negatively dependent on soil nitrate (Figure 4-2) on the basis of two separate multiple regressions. Tilman et al. is demonstrating the very instability in model parameterization caused by the inclusion of multiple collinear variables and instead of recognizing this, the authors draw two rather unlikely conclusions from their data.

First, they conclude that while species richness decreases soil nitrate by increasing nitrogen uptake, soil nitrogen uptake is unrelated to plant productivity. Then they conclude that increasing soil nitrate decreases plant productivity. They do all this rather than draw what I view to be the much more likely explanation—that is, (1) that increasing plant productivity—regardless of species richness—decreases soil nitrate by increasing nitrogen uptake, and (2) multiple regression is unable to correctly partition variability between multiple collinear input variables.

This study typifies the main issue with the majority of diversity-nutrient retention studies. The majority of studies evaluating the effects of plant mixture diversity on nitrogen retention are designed in such a way that plant productivity co-varies with diversity and the issue is either left unaddressed or is inappropriately addressed (Ewel et al., 1991; Oelmann et al., 2011; Scherer-Lorenzen et al., 2003; Symstad et al., 1998; Tilman et al., 1996). Consequently, it is unclear whether nitrogen retention is genuinely related to species richness or simply to plant productivity. Of the studies I reviewed, only Hooper and Vitousek (1998) held productivity constant while varying plant mixture diversity, and in that study plant mixture diversity was found to be unrelated to nitrogen retention.

Much like the cover cropping literature, the literature looking at the effect of plant mixture diversity on nutrient retention focuses overwhelmingly on nitrogen. However, there are a couple studies that have looked at phosphorus. Those studies have held either biomass productivity or planting density constant and observed no relationship between soil phosphorus retention and plant mixture diversity (Hooper and Vitousek, 1998; Zhang et al., 2010).

Agricultural systems are known for being more “leaky” of nutrients than their natural system counterparts (Swift and Anderson, 1994). Given the sparseness and weakness of the literature on the subject of plant mixture diversity on soil nutrient retention—particularly those nutrients that are not nitrogen, and the importance of understanding how to manage nutrient dynamics in agricultural fields, the goal of this study was to evaluate the effect of cover crop mixture diversity on soil nutrient retention.

MATERIALS AND METHODS

Research site

The research site (41°40'15"N 96°33'45"W) was located in Hooper, Nebraska on a family farm that had been managed continuously for the past 30 years with regular applications of manure being used to manage soil fertility. The site was level (0-2% slopes), with fairly little soil nutrient loss expected due to erosion. The soil was a Moody silty clay loam (fine-silty, mixed, superactive, mesic Udic Haplustoll). All depths sampled fell into the textural class of silty clay loam. Soil chemical characteristics for the site based on the control plots are provided in Table 4-1.

Table 4-1. Soil chemical characteristics (±SEM) based on control plots (N=4).

Depth	pH	Total C	Total N	NO_3-N	Mehlich-P	K^+	SO_4-S	Cl^-
(cm)	(1:1 H_2O)	------------(%)------------		-------------------------(mg·kg soil^{-1})-------------------------				
0-10	6.2±0.1	2.4±0.8	0.205±0.003	56±5	158±15	670±35	11±1.0	5.9±0.3
10-20	5.9±0.1	1.8±0.1	0.155±0.005	14±2	87±18	326±19	6.6±0.6	4.5±0.5
20-30	5.4±0.1	1.7±0.2	0.140±0.012	15±2	69±16	245±19	5.1±0.4	6.0±0.3
30-60	6.1±0.1	1.2±0.1	0.118±0.008	20±4	46±8	231±25	6.0±1.1	6.8±0.8

This site was a part of a larger study that included ten other sites. This site was selected for further study on the relationship between cover crop mixture diversity on soil nutrient retention on the basis that it had substantial cover crop establishment but minimal weed presence. The other ten sites either had either modest cover crop establishment or substantial cover crop establishment with relatively high levels of weed biomass.

Experimental design

Cover crop treatments were planted on August 31, 2013. Details regarding the twenty cover crop treatments used in this study and their establishment can be found in Chapter 2.

Plant sampling

Plant aboveground biomass samples were taken October 31, 2013 prior to winterkill. Biomass was sampled using two randomly placed quadrats ($0.18\ m^2$) in each plot. Both cover crop and weed species were cut at the soil surface, separated by species and dried at 55°C for 7 days and weighed to determine dry biomass.

Soil sampling and laboratory analysis

Soils were sampled on April 9, 2014 prior to the planting of corn. Every plot was sampled from 0-10 cm with treatments 1, 11, 12, 13, and 17 additionally being sampled from 10-20, 20-30, and 30-60 cm. Each sample was a composite of five cores (3.2 cm diameter). Samples were oven dried at 60˚C for at least 24 hours and analyzed for extractable nitrogen (NO_3-N), phosphorus (P), potassium (K), sulfate, (SO_4-S) and chloride (Cl). Control plot samples were additionally evaluated for soil texture, total carbon, total nitrogen, and pH to help characterize the site (Table 4-1).

Soil NO_3-N was extracted with potassium chloride. Soil P was extracted with the Mehlich-3. K was extracted with ammonium acetate. SO_4-S was extracted with calcium phosphate. Cl was extracted with calcium nitrate. Soil texture was determined using the hydrometer method. Total carbon and nitrogen were determined through dry combustion, and pH was determined in a 1:1 mixture with water. Soil chemical analyses were conducted using the procedures recommended by NCERA-13 (2015).

Data analysis

Cover crop treatment effect sizes were calculated for each nutrient at each sampled depth by the following equation:

$$\text{Effect size (\%)} = \frac{C_{treatment} - C_{control}}{C_{control}} * 100$$

Where $C_{treatment}$ is the nutrient concentration of the treatment plot and $C_{control}$ is the nutrient concentration of the corresponding no cover control plot in that block and for that depth.

Soil nutrient concentrations at each depth were regressed against total aboveground plant biomass using ordinary least squares regression. Total plant biomass values included a small amount of weed biomass (a maximum of 43 g m^{-2}) in the form of volunteer winter wheat in addition to cover crop biomass.

To see if increasing plant mixture diversity increased nutrient retention, I tested whether the slope of the relationship between soil nutrient concentration in the top 10 cm and total aboveground plant biomass was positively affected by cover crop species richness (Figure 4-3a).

To see if plant mixtures retain more nutrients than plant monocultures, I tested whether the slope of this relationship was greater for the cover crop mixtures than the cover crop monocultures (Figure 4-3b). These approaches were used to control for the relationship between total aboveground plant biomass on soil nutrient concentration. All statistical analyses were conducted using R 3.1.0 (R Core Team, 2014).

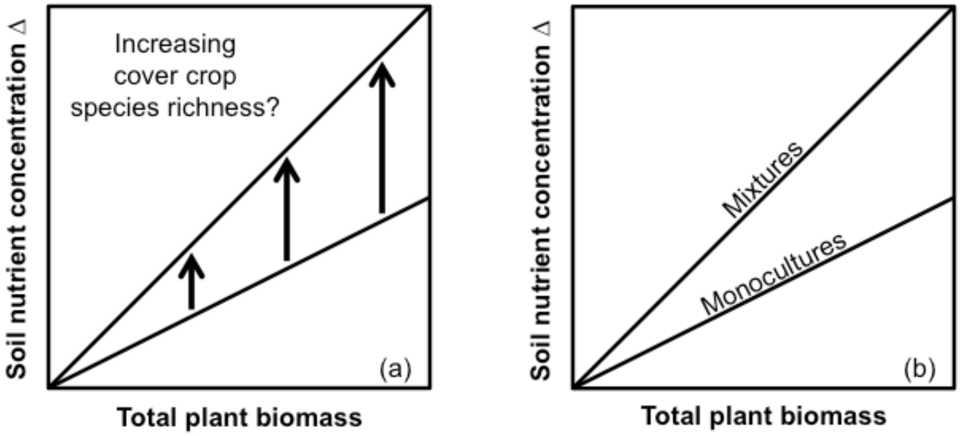

Figure 4-3. Hypothesized effects of (a) increasing cover crop mixture species richness and (b) mixing plants on the relationship between soil nutrient concentration changes and plant biomass.

Given that there were small amounts of winter wheat in some of the plots, it might be argued that it would be better to conduct this analysis with total plant species richness rather than cover crop mixture species richness. While I decided to use cover crop species richness because I believe this approach yields results of most interest cover crop management, I would like to note that using total plant species richness in the analysis yields the same interpretative conclusion presented here.

In the interpretations that follow I assume that the majority of the nutrients in the aboveground biomass have been returned to the soil by the time of soil sampling. This assumption is made on the basis of two pieces of evidence. First, at the time of soil sampling, the cover crop residue was mostly degraded. Second, if we assume constant bulk density throughout the soil profile—a reasonable assumption for this soil series (National Cooperative Soil Survey, 2016)—we find that there is no significant difference in the total amount of soil extractable nutrients between the cover crop plots and the control plots in the top 60 cm, just a difference in the distribution of those nutrients, suggesting that whatever nutrients were taken up by the cover crop were returned to the soil by the time of soil sampling.

RESULTS

Cover cropping and nutrient retention

The presence of a fall cover crop increased the concentrations of soil extractable NO_3-N, K, SO_4-S, and Cl in the upper 10 centimeters of the soil profile in the spring as compared to the control plots (Figure 4-4). Soil extractable NO_3-N, K, SO_4-S, and Cl concentrations were increased by an average of 70, 15, 37, and 91%, respectively. Soil P concentrations were not consistently or significantly affected by cover cropping.

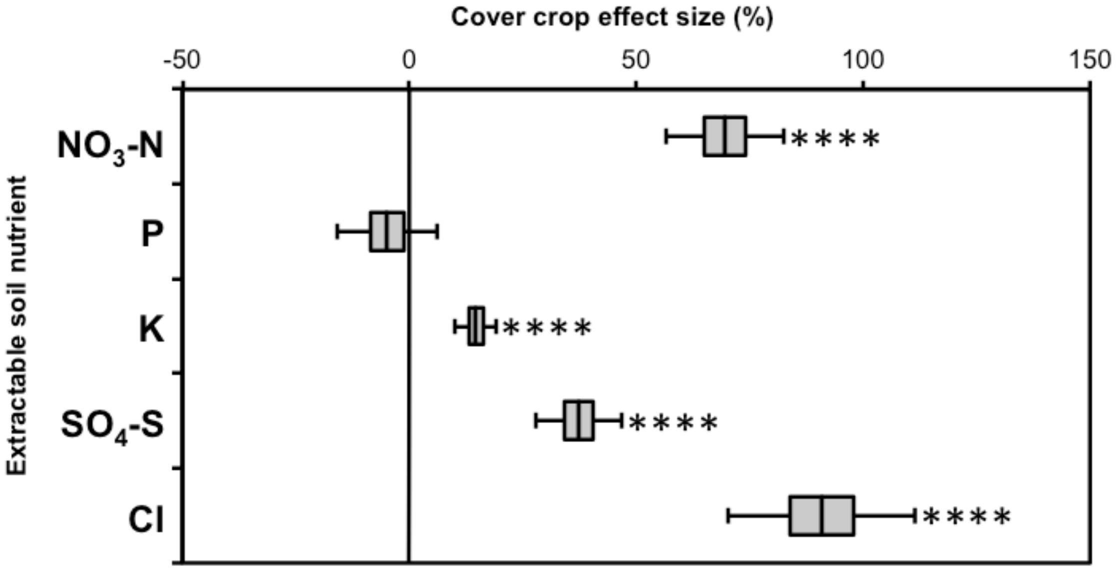

Figure 4-4. Mean effect size of cover cropping on extractable soil nutrient concentrations from 0-10 cm (N=76). Boxes and bars represent 50% and 95% confidence intervals, respectively. H_0: $\mu = 0$; H_a: $\mu \neq 0$. *P*-value < 0.05(*); < 0.01(**); < 0.001(***); < 0.0001(****).

Cover cropping increased soil extractable NO_3-N, SO_4-S, and Cl concentrations in the top 10 cm of the soil profile but decreased their concentrations in the lower parts of the soil profile—suggesting that the cover crops helped to prevent these nutrients from leaching into the soil profile. Soil extractable K concentrations, however, were increased throughout the soil profile under the cover crops—suggesting perhaps that cover cropping weathered mineral K into extractable forms throughout the soil profile as well as decreased K leaching (Rich, 1968). P concentrations and distributions were not significantly affected by cover cropping (Figure 4-5).

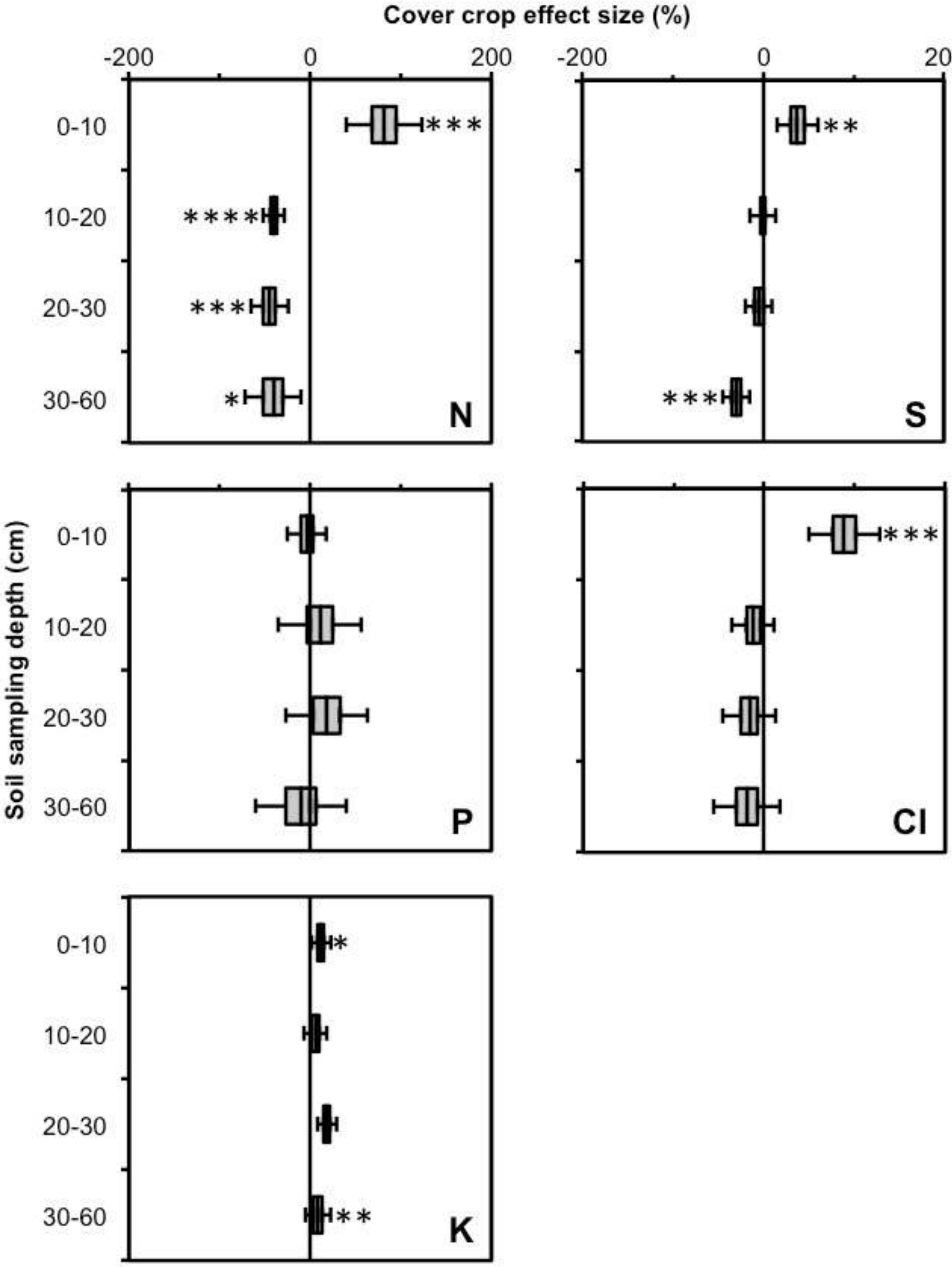

Figure 4-5. Mean effect size of cover cropping on extractable soil nutrient concentrations from 0-10, 10-20, 20-30, and 30-60 cm (N=16). Boxes and bars represent 50% and 95% confidence intervals, respectively. H_0: $\mu = 0$; H_a: $\mu \neq 0$. *P*-value < 0.05(*); < 0.01(**); < 0.001(***); < 0.0001(****).

Total aboveground plant biomass and nutrient retention

Not only were NO_3-N, SO_4-S, and Cl concentrations increased in the upper 10 cm by the presence of cover crops, that increase was positively related to total aboveground plant biomass put on in the fall (Table 4-2; Figure 4-6).

Table 4-2. Parameter estimates for linear models relating soil extractable nutrient concentrations to total aboveground plant biomass by sampling depth.

Nutrient	Depth (cm)[†]	Intercept±SEM	Slope±SEM[‡]	*p*-value
NO_3-N	0-10	81±6	$0.05±0.02^{*}$	0.0498
	10-20	12±1	$-0.018±0.005^{**}$	0.0017
	20-30	15±2	$-0.032±0.010^{**}$	0.0041
	30-60	19±2	$-0.042±0.011^{**}$	0.0014
P	0-10	152±13	$-0.03±0.06^{NS}$	0.58
	10-20	82±14	$-0.03±0.07^{NS}$	0.71
	20-30	63±12	$0.02±0.06^{NS}$	0.75
	30-60	36±8	$-0.01±0.03^{NS}$	0.85
K	0-10	713±30	$0.26±0.13^{NS}$	0.06[§]
	10-20	361±33	$-0.1±0.2^{NS}$	0.54
	20-30	292±20	$-0.1±0.1^{NS}$	0.49
	30-60	246±13	$-0.04±0.06^{NS}$	0.53
SO_4-S	0-10	12.4±0.8	$0.009±0.004^{*}$	0.02
	10-20	6.4±0.6	$-0.001±0.003^{NS}$	0.78
	20-30	5.2±0.4	$-0.003±0.002^{NS}$	0.24
	30-60	5.1±0.5	$-0.005±0.003^{NS}$	0.07
Cl	0-10	6.5±1.0	$0.024±0.005^{****}$	<0.0001
	10-20	4.4±0.5	$-0.003±0.002^{NS}$	0.18
	20-30	7.1±0.8	$-0.012±0.004^{**}$	0.0048
	30-60	11±3	$-0.02±0.01^{NS}$	0.15

[†] df = 78 for 0-10 cm depth and 18 for 10-20, 20-30, and 30-60 cm depths.

[‡] Superscripts indicate *p*-values for the following hypothesis test—H_0: slope = 0; H_a: slope ≠ 0. *P*-value >0.05(^{NS}); < 0.05(*); < 0.01(**); < 0.001(***); < 0.0001(****). Adjacent column indicates exact *p*-values.

[§] The 0-10 cm depth for block 4 was enriched in potassium compared to blocks 1-3. Including a block effect in this model pushes this p-value to 0.02 and into our threshold for significance.

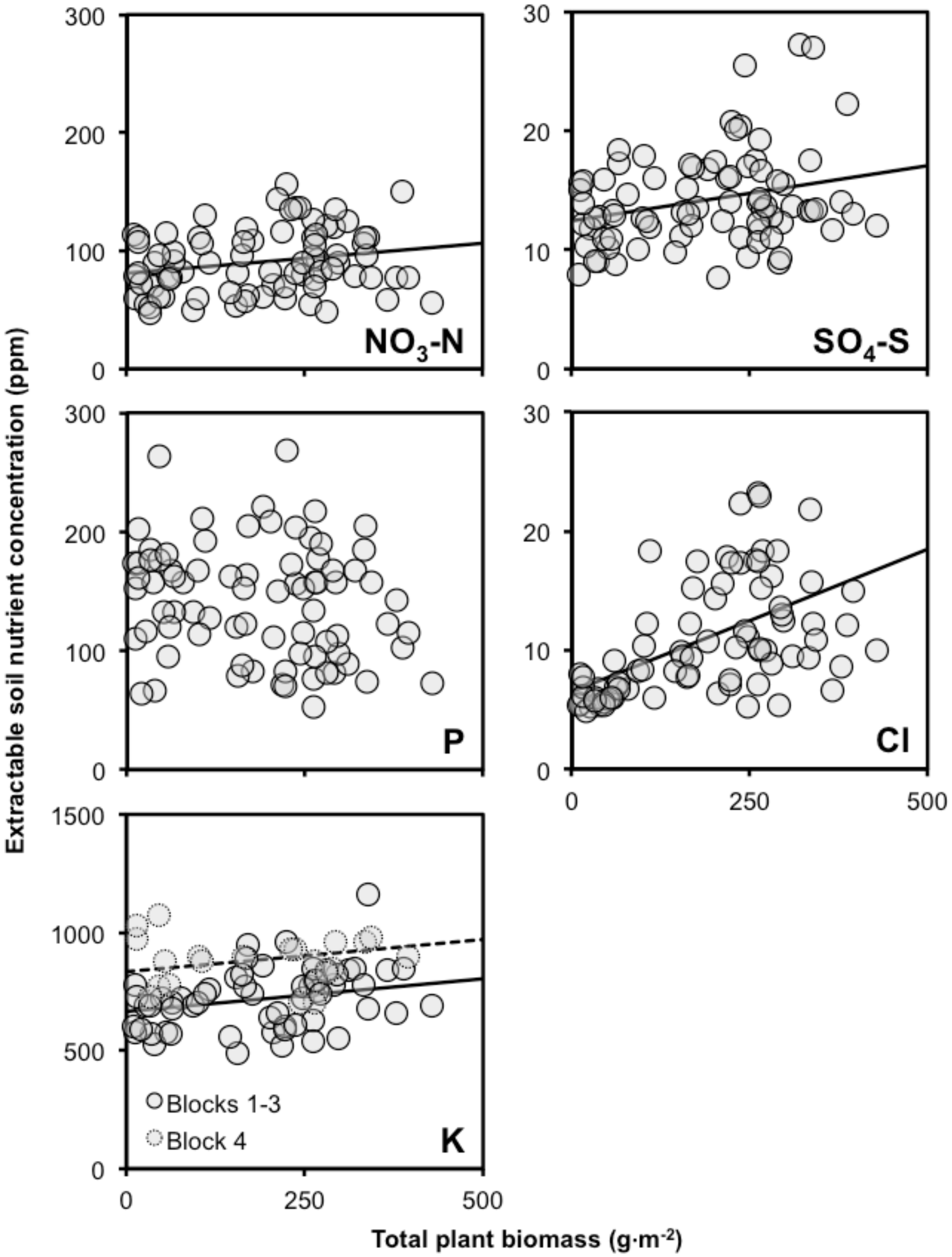

Figure 4-6. Extractable nutrient concentrations, 0-10 cm, versus total aboveground plant biomass with linear regressions plotted when slope parameter significantly different from zero at $\alpha = 0.05$.

Furthermore, with regards to NO_3-N and Cl, many of the lower depths sampled had a significant negative relationship with total plant biomass. That is, increasing total aboveground plant biomass was associated with decreased soil extractable nutrient concentrations in the lower depths. While there were no significant relationships observed between SO_4-S concentrations in the lower depths sampled and plant biomass, all the slope parameter estimates relating these two variables were at the least negative, if not statistically significant at the $\alpha = 0.05$ level. Taken together, these observations are all consistent with the idea that cover cropping helps to prevent nutrient leaching through nutrient uptake—with greater cover crop biomass being associated with greater soil nutrient retention.

Soil extractable K concentrations in the upper 10 cm were also positively related to total plant biomass, which is consistent with both the idea that cover cropping can help prevent nutrient leaching and the idea that cover cropping can weather mineral forms of K into soil extractable forms (Figure 4-6). However, if we look at the relationship between K in the lower depths sampled and total plant biomass, we find that there are no statistically significant relationships. All of the slope estimates are negative suggesting that as total aboveground plant biomass increases, soil extractable K in the lower depths decreases weakly, which is more consistent with the idea that cover cropping might help prevent small amounts of K leaching but not with the idea that cover cropping can weather mineral K into extractable K (Table 4-2). I hypothesize that both mechanisms are at work and therefore dampening the effects of one another.

Soil extractable P which was not significantly affected by cover cropping was also not significantly affected by total aboveground plant biomass at any of the depths sampled. Furthermore, the sign on the slope values relating P to total aboveground plant biomass was inconsistent across soil depths.

Cover crop diversity and soil nutrient retention

To test the hypothesis that increased plant mixture diversity increases soil nutrient retention, I tested whether adding an interaction term between total plant species richness and total aboveground plant biomass significantly improved the linear models relating extractable nutrient concentrations in the upper 10 cm to total plant biomass. The results of this analysis are summarized in Table 4-3. None of the nutrient models were improved by the inclusion of this interaction term.

Table 4-3. Parameter estimates for linear models relating soil nutrient concentration in the 0-10 cm depth (NC) for soil extractable NO_3-N, P, K, SO_4-S, and Cl to total aboveground plant biomass (BIOM) with cover crop species richness (SR) interacting with biomass (df = 78).

Nutrient	**Equation**	**Parameter**†	**Estimate±SEM**‡	***p*-value**
NO_3-N	NC ~ BIOM + BIOM:SR	BIOM	$0.05\pm0.02^{*}$	0.01
		BIOM:SR	-0.001 ± 0.005^{NS}	0.87
P	NC ~ BIOM + BIOM:SR	BIOM	0.01 ± 0.05^{NS}	0.78
		BIOM:SR	-0.01 ± 0.01^{NS}	0.23
K	NC ~ BIOM + BIOM:SR	BIOM	$0.42\pm0.11^{***}$	0.0002
		BIOM:SR	-0.05 ± 0.03^{NS}	0.06
SO_4-S	NC ~ BIOM + BIOM:SR	BIOM	$0.011\pm0.003^{***}$	0.0008
		BIOM:SR	-0.0005 ± 0.0008^{NS}	0.51
Cl	NC ~ BIOM + BIOM:SR	BIOM	$0.026\pm0.004^{****}$	<0.0001
		BIOM:SR	-0.001 ± 0.001^{NS}	0.48

† Intercepts fixed to intercept value from global model fit (N = 80).

‡ Superscripts indicate *p*-values for the following hypothesis test—H_0: slope = 0; H_a: slope ≠ 0. *P*-value >0.05(^{NS}); < 0.05(*); < 0.01(**); < 0.001(***); < 0.0001(****). Adjacent column indicates exact *p*-values.

To test the related hypothesis that plant mixtures retain more soil nutrients than plant monocultures, I evaluated whether the slope of the relationship between soil nutrient concentrations in the upper 10 cm and total aboveground plant biomass was greater for the cover crop mixtures than the cover crop monocultures. The results of this analysis are summarized in Table 4-4. An F-test was used to compare the monoculture models with the mixture models. There was no significant difference between the monoculture models and the mixture models for any of the nutrients tested.

Table 4-4. Slope estimates for linear models relating soil extractable NO_3-N, P, K, SO_4-S, and Cl in the 0-10 cm depth to total aboveground plant biomass for cover crop monocultures (df = 36) and cover crop mixtures (df = 40) with F-test results.

Nutrient	Cover crop group	Slope±SEM†‡	F-value	p-value
NO_3-N	Monocultures	$0.046 \pm 0.017^{**}$	0.61	0.44
	Mixtures	$0.056 \pm 0.020^{**}$		
P	Monocultures	-0.014 ± 0.051^{NS}	0.40	0.53
	Mixtures	-0.046 ± 0.036^{NS}		
K	Monocultures	$0.358 \pm 0.111^{**}$	2.39	0.13
	Mixtures	$0.185 \pm 0.092^{*}$		
SO_4-S	Monocultures	$0.012 \pm 0.003^{***}$	1.16	0.28
	Mixtures	$0.008 \pm 0.002^{**}$		
Cl	Monocultures	$0.023 \pm 0.003^{****}$	1.30	0.26
	Mixtures	$0.025 \pm 0.003^{****}$		

† Intercepts fixed to intercept value from global model fit (N = 80).
‡ Superscripts indicate *p*-values for the following hypothesis test—H_0: slope = 0; H_a: slope ≠ 0. *P*-value > 0.05(^{NS}); < 0.05(*); < 0.01(**); < 0.001(***); < 0.0001(****).

DISCUSSION

Cover crop management conclusions

Cover cropping can help to prevent NO_3-N, K, SO_4-S, and Cl from leaching losses. Cover cropping may also help to release mineral K into soil extractable forms. While increasing cover crop biomass can increase the magnitude of these effects, I found no evidence that increasing plant mixture diversity increases the magnitude of these effects independent of its effects on biomass. Nor did I find that mixtures help to retain soil nutrients better than monocultures once variations in biomass were accounted for. In managing cover crops for increased nutrient retention, I found the key to be increasing cover crop biomass rather than cover crop species richness.

Parting thoughts regarding diversity and soil nutrient retention

The findings of this study extend beyond agronomic applications, and help us to address the question of whether increased diversity equates to resource use efficiency—an idea that underpins both the diversity-productivity and diversity-invasibility hypotheses which were discussed in Chapters 2 and 3, respectively.

In the introduction, I discussed the issues with interpreting studies where plant productivity co-varies with diversity. In this study, plant productivity also co-varies with diversity (Pearson's correlation coefficient = 0.31, *p*-value = 0.007), but I attempt to tease apart the effects of diversity and productivity in the by first characterizing the effect of productivity on soil nutrient concentrations. I conclude that effects of vegetation on soil nutrient retention are often governed by plant biomass, finding no evidence that species richness has an effect on soil nutrient retention independent of its relationship with biomass. As with the invasibility study discussed in Chapter 3, we should be cautious in interpreting correlations between diversity and various other metrics as causation. More often than not, I suspect that biomass productivity is the true mediator of these effects. To highlight this issue, I would like to end this chapter by casting past studies relating diversity, productivity, and nutrient cycling in a different light than they were presented.

In a study by van Ruijven and Berendse (2005), the authors conduct a four-year plant mixture diversity study in which the positive slope of the relationship between diversity and productivity increased over time. They used this as evidence of increased resource use efficiency of mixtures. I propose an alternate interpretation of their results. I suggest that what actually might be happening is that an initial mild diversity-productivity correlation is being strengthened each year as the productive, high diversity treatments enrich the local soil fertility as compared to the unproductive, low diversity treatments. Thus, as years pass, the high diversity treatments are being grown on increasingly more fertile soil, strengthening the positive correlation between diversity and productivity. Consider that Oelmann et al. (2011) found that the positive relationship between plant diversity and soil nitrogen storage tended to increase over time and that this increase was primarily driven by variations in biomass.

Consider also that when Dybzinski et al. (2008) grew *Echinacea purpurea* in soil that had been growing grassland vegetation of varying levels of species richness for ten years, they found that the plants produced more biomass on soil “trained” under greater levels of species richness. They attributed this to the increased nitrogen content of the soils under the more diverse grassland treatments—further concluding that more diverse systems retain and add greater amounts of nutrients in the soil. However, in the grassland experiment where they obtained their soils, productivity co-varied with richness. Thus, it could have been due to increased productivity, rather than increased diversity, that a greater amount of soil nutrients were held in the soil.

Fornara and Tilman (2009) noted that after 13 years more diverse plots had greater soil nitrogen levels. They concluded that this increase in soil nitrogen contributed to the productivity of the diverse plots over time and that these increases in soil nitrogen were mediated by plant diversity. While I agree with the first assertion that increases in soil nitrogen helped to increase the productivity of the diverse plots in later years of the study, I disagree that these increases in soil nitrogen were mediated by plant diversity. Rather, I propose that these increases in soil nitrogen were mediated by plant productivity.

Spehn et al. (2005) and Fargione et al. (2007) both concluded that one of the reasons diverse mixtures are more productive on average is because diverse mixtures are more resource use efficient. They both base their conclusions on the observation that in their respective studies, plant mixtures contained on average more aboveground biomass nitrogen than monocultures. I assert that this is not evidence of increased resource use efficiency of mixtures but simply evidence that more productive treatments inevitably have greater amounts of total nutrients in their biomass. Productive monocultures would also be expected to have large amounts of aboveground biomass nitrogen. This expectation is confirmed by a meta-analysis by Cardinale et al. (2006) that found that the resource use of the most species-rich mixtures was no different than the most productive species used in each experiment.

In these studies, was it really that more diverse mixtures were more resource use efficient? Or was it that in these studies, more diverse treatments were on average more productive and productive treatments, by definition, take up and contain more nutrients? I propose that we revisit these studies and characterize and control for the relationship between biomass on nutrient capture before characterizing the relationship between diversity and nutrient capture.

References

Allison, F. E., E. M. Roller, and J. E. Adams. 1959. Soil fertility studies in lysimeters containing lakeland sand. USDA Tech. Bull. 1199:1-62.

Askegaard, M., J. E. Olesen, I. A. Rasmussen, and K. Kristensen. 2011. Nitrate leaching from organic arable crop rotations is mostly determined by autumn field management. Agr. Ecosyst. Environ. 142:149-160.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecol. Lett. 9(10):1146-1156.

Bertsch, P. M., and G.W. Thomas. 1985. Potassium status of temperate region soils. In: R. D. Munson, editor, Potassium in agriculture. American Society of Agronomy, Madison, WI. p. 131-162.

Brandi-Dohrn, F. M., R. P. Dick, M. Hess, S. M. Kauffman, D. D. Hemphill, and J. S. Selker. 1997. Nitrate leaching under a cereal rye cover crop. J. Environ. Qual. 26:181-188.

Bray, R. H. 1954. A nutrient mobility concept of soil-plant relationships. Soil Sci. 78(1):9-22.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443(26):989-992.

Culley, J. L. B, E. F. Bolton, and V. Bernyk. Suspended solids and phosphorus loads from a clay soil: I. Plot studies. J. Environ. Qual. 12:493-498.

Djodjic, F., K. Börling, and L. Berström. 2004. Phosphorus Leaching in Relation to Soil Type and Soil Phosphorus Content. J. Environ. Qual. 33:678-684.

Dybzinski, R., J. E. Fargione, D. R. Zak, D. Fornara, and D. Tilman. 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. Oecologia 158:85-93.

Eriksen, J., and K. Thorup-Kristensen. 2002. The effect of catch crops on sulphate leaching and availability of S in the succeeding crop on sandy loam soil in Denmark. Agr. Ecosyst. Environ. 90:247-254.

Ewel, J.J., M.J. Mazzarino, and C.W. Berish. 1991. Tropical soil fertility changes under monocultures and successional communities of different structure. Ecol. Appl. 1:289-302.

Fargione, J., D. Tilman, R. Dybzinski, J. H. R. Lambers, C. Clark, W. S. Harpole, J. M H. Knops, P. B. Reich, and M. Loreau. 2007. From selection to complementarity: shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. Proc. R. Soc. B 274:871-876.

Fornara, D. A., and D. Tilman. 2009. Ecological Mechanisms Associated with the Positive Diversity—Productivity Relationship in an N-Limited Grassland. Ecology 90(2):408-418.

Graham, M. H. 2003. Confronting multicollinearity in ecological multiple regression Ecology 84(11):2809-2815.

Hooper, D. U., and P. M. Vitousek. 1998. Effects of Plant Composition and Diversity on Nutrient Cycling. Ecol. Monogr. 68(1):121-149.

Isse, A., MacKenzie, A., Stewart, K., Cloutier, D., and Smith, D. 1999. Cover crops and nutrient retention for subsequent sweet corn production. Agron. J. 91:934–939.

Kauffman, S. J., D. L. Royer, S. Chang, and R. A. Berner. 2003. Export of chloride after clear-cutting in the Hubbard Brook sandbox experiment. Biogeochemistry 63:23-33.

Kirchmann, H., A. E. Johnny Johnston, and L. F. Bergström. 2002. Possibilities for Reducing Nitrate Leaching from Agricultural Land. Ambio 31(5):404-408.

Lewan, E. 1994. Effects of a catch crop on leaching of nitrogen from a sandy soil: simulations and measurements. Plant Soil 166:137-152.

Martinez, J., and G. Guiraud. 1990. A lysimeter study of the effects of a ryegrass catch crop, during a winter wheat/maize rotation, on nitrate leaching and on the following crop. J. Soil Sci. 41:5-16.

McLenaghen, R. D., K. C. Cameron, N. H. Lampkin, M. L. Daley, and B. Deo. 1996. Nitrate leaching from ploughed pasture and the effectiveness of winter catch crops in reducing leaching losses, New Zeal. J. Agr. Res. 39(3):413-420.

Meisinger, J. J., W. L. Hargrove, R. L. Mikkelsen, J. R. Williams, and V. W. Benson. 1991. Effects of cover crops on groundwater quality. In: W. L. Hargrove, editor, Cover Crops for Clean Water. Soil and Water Conservation Society, Ankeny, IA. p. 57-68.

Milburn, P., J. A. MacLeod, and S. Sanderson. 1997. Control of fall nitrate leaching from early harvested potatoes on Prince Edward Island. Can. Agr. Eng. 39:263-271.

Mueller, K. E., D. Tilman, D. A. Fornara, and S. E. Hobbie. 2013. Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. Ecology 94(4):787-793.

National Cooperative Soil Survey. Moody Series. NCSSL Characterization Database. http://ncsslabdatamart.sc.egov.usda.gov/ (accessed February 14, 2016).

NCERA-13. 2015. Recommended Chemical Soil Test Procedures for the North Central Region. North Central Regional Research Publication No. 221.

Nolan, C. W., and W. C. Pritchett. 1960. Certain factors affecting the leaching of potassium from sandy soils. Proc. Soil Crop Sci. Soc. Fla. 20:139-145.

Oelmann, Y., N. Buchmann, G. Gleixner, M. Habekost, C. Roscher, S. Rosenkranz, E-D. Schulze, S. Steinbeiss, V. M. Temperton, A. Weigelt, W. W. Weisser, and W. Wilcke. 2011. Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment. Global Biogeochem. Cy. 25. GB2014. doi:10.1029/2010GB003869.

Poudel, D.D., W. R. Horwath, J.P. Mitchell, and S. R. Temple. 2001. Impacts of cropping systems on soil nitrogen storage and loss. Agr. Syst. 68:253-268.

Quémener, J. 1986. Important factors in potassium balance sheets. In: Nutrient balances and the need for potassium. Proceedings of the 13th IPI Congress. Reim, France. Aug. 1986. International Potash Institute, Bern, Switzerland. p. 41-72.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rich, C.I. 1968. Mineralogy of soil potassium. In: V.J. Kilmer, S. E., Younts, N. C. Brady, editors, The role of potassium in agriculture. American Society of Agronomy, Madison, WI. p. 79-91.

Scherer-Lorenzen, M., C. Palmborg, A. Prinz, and E-D. Schulze. 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84(6):1539-1552.

Shepard, M. A. 1999. The effectiveness of cover crops during eight years of a UK sandland rotation. Soil Use Manage. 15:41-48.

Shepherd, M. A., and E. I. Lord. 1996. Nitrate leaching from a sandy soil—The effect of previous crop and post-harvest soil management in an arable rotation. J. Agr. Sci. 127:215-229.

Sørensen, J. N. 1991. Effect of catch crops on the content of soil mineral nitrogen before and after winter leaching. Z. Pflanzenernahr. Bodenk. 155:61-66.

Spehn, W. M., A. Hector, J. Joshi, M. Scherer-Lorenzen, B. Schmid, E. Bazeley-White, C. Beierkuhnlein, M. C. Caldeira, M. Diember, P. G. Dimitrakopoulos, J. A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Högberg, K. Huss-Danell, A. Jumpponen, J. Koricheva, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, C. Palmborg, J. S. Pereira, A. B. Pfisterer, A. Prinz, D. J. Read, E.-D. Schulze, A.-S. D. Siamantziouras, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 2005. Ecosystem effects of biodiversity manipulations in European Grasslands. Ecol. Monogr. 75(1):37-63.

Strock, J. S., P. M. Porter, and M. P. Russelle. 2004. Cover Cropping to Reduce Nitrate Loss through Subsurface Drainage in the Northern U. S. Corn Belt. J. Environ. Qual. 33:1010-1016.

Swift, M. J., and J. M. Anderson. 1994. Biodiversity and ecosystem function in agricultural systems. In: E. D. Schulze, and H. A. Mooney, editors, Biodiversity and Ecosystem Function. Springer-Verlag, Berlin, Germany. p. 15-41.

Symstad, A. J., D. Tilman, J. Willson, and J. M. H. Knops. 1998. Species Loss and Ecosystem Functioning: Effects of Species Identity and Community Composition. Oikos 81(2):389-397.

Thomsen, I. K. 2005. Nitrate leaching under spring barley is influenced by the presence of a ryegrass catch crop: Results from a lysimeter experiment. Agr. Ecosyst. Environ. 111:21-29.

Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature 379:718-720.

Tilman, D., J. Knops, D. Wedin, and P. Reich. 2001. Experimental and Observational Studies of Diversity, Productivity, and Stability. In: A. P. Kinzig, D. Tilman, and S. Pacala, editors, The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions. Princeton Univ. Press, Princeton, NJ. p. 42-70.

Truog, E., and R.J. Jones. 1938. The fate of soluble potash applied to soils. Ind. Eng. Chem. 30:882-885.

Turtola, E., and A. Jaakkola. 1995. Loss of phosphorus by surface runoff and leaching from a heavy clay soil under barley and grass ley in Finland. Acta Agr. Scand. B-S P. 45:158-165.

van Ruijven, J., and F. Berendse. 2005. Diversity-productivity relationships: initial effects, long-term patterns, and underlying mechanisms. P. Natl. A. Sci. USA 102:695-700.

Vitousek, P.M., and D.U. Hooper. 1994. Biological Diversity and Terrestrial Ecosystem Biogeochemistry. In: E-D. Schulze, and H.A. Mooney, editors, Biodiversity and Ecosystem Function. Springer-Verlag, Berlin, Germany. p. 3-14.

Weinert, T.L., W. L. Pan, M.R. Moneymaker, G. S. Santo, and R. G. Stevens. 2002. Nitrogen recycling by nonleguminous winter cover crops to reduce leaching in potato rotations. Agron. J. 94:365–372.

Wyland, L. J., L. E. Jackson, W. E. Chaney, K. Klonsky, S. T. Koike, and B. Kimple. 1996. Winter cover crops in vegetable cropping system: Impacts on nitrate leaching, soil water, crop yield, pests and management costs. Agr. Ecosyst. Environ. 59:1-17.

Zhang, F., and L. Li. 2003. Using competitive and facilitative interactions in intercropping systems enhances crop productivity and nutrient use efficiency. Plant Soil. 248:305-312.

Chapter 5 - Cover crop mixture diversity and soil microbial biomass and community structure

ABSTRACT

Soil microbial biomass and community structure are affected by plant growth but it's unclear whether these parameters are affected by plant mixture diversity. This study was conducted to determine the effects of cover crop mixture species richness and functional richness on soil microbial biomass and community structure. Nine cover crop species representing three functional groups were used in this study—grasses (barley, oats, wheat), legumes (Austrian winter pea, red clover, yellow blossom sweetclover), and brassicas (radish, rapeseed, turnip). Twenty treatments reflecting varying levels of cover crop species and functional richness were replicated four times in a harvested wheat field in Hooper, NE. All nine cover crop species were planted in monoculture and the most diverse mixture used contained all nine of these species. Cover crops were planted on August 31, 2013 and sampled for aboveground biomass on October 31, 2013 prior to winterkill. Soil samples were taken from 0-10 cm in all plots on April 9, 2014 for fatty acid methyl esters (FAMEs) extraction. Soil microbial biomass was estimated by the total FAMEs extracted and soil microbial community structure was characterized by individual FAMEs extracted. Cover cropping was associated with an increase in soil microbial biomass and alterations in soil microbial community structure with total plant productivity being a significant determinant of the size of these increases and alterations. There was no evidence, however, that cover crop species richness or functional richness predictably altered soil microbial biomass or community structure outside of their probabilistic effects on plant biomass.

INTRODUCTION

Vegetated soils typically have greater soil microbial biomass than un-vegetated soils as well as altered soil microbial community structures as compared to bare soils (Bernard et al., 2012; Buyer et al., 2010; Carrera et al., 2007; Drijber et al., 2000; Fang et al., 2001; Ibekwe and Kennedy, 1998; Larkin, 2008; Lehman et al., 2014; Lehman et al., 2012; Mendes et al., 2004; Schutter and Dick, 2002; Schutter et al., 2001). It's not clear though, whether or not plant mixture diversity predictably affects soil microbial biomass or community structure.

Some authors have observed a positive correlation between plant mixture diversity and soil microbial biomass metrics (Carney and Matson, 2004; Chung et al., 2007; De Deyn et al., 2011; Eisenhauer et al., 2010; Guenay et al., 2013; Spehn et al., 2000; Stephen et al., 2000; Zak et al., 2003) while others have observed more idiosyncratic effects of mixing plants on soil microbial biomass (Habekost et al., 2008; Wardle and Nicholson, 1996; Wortman et al., 2013). The literature on the effect of plant mixture diversity on soil microbial community structure is even less clear with some authors observing that increasing plant mixture diversity does alter soil microbial community structure (Carney and Matson, 2004) and others not observing an effect (Wortman et al., 2013; Zhang et al., 2010).

While the hypotheses that increasing plant mixture diversity should (1) increase soil microbial biomass and (2) alter soil microbial community structure have been tested in many places, these hypotheses are rarely formally named. Following the lead of Chapman and Newman (2010), however, I will refer to these hypotheses as the diversity-increased abundance and diversity-altered microbial community hypotheses.

The goal of this study was to test these two hypotheses in the context of cover crop mixtures—asking the questions of whether increasing cover crop mixture species or functional richness (1) increases soil microbial biomass and (2) predictably alters soil microbial community structure.

MATERIALS AND METHODS

Research site

The research site (41°40'15"N 96°33'45"W) was located in Hooper, Nebraska on a family farm under a corn-soybean-wheat rotation. The farm had been managed continuously for the past 30 years with regular applications of manure to manage soil fertility and a combination of cultivation and banded herbicide to manage weeds. The farm manager had recently begun to experiment with cover crops planted after winter wheat harvest—using a combination of oats, various brassicas, and various cool-season legumes. The soil was a Moody silty clay loam (fine-silty, mixed, superactive, mesic Udic Haplustoll).

Experimental design

Details regarding the twenty cover crop treatments used in this study, their establishment, and their biomass sampling can be found in Chapter 2.

Soil sampling and preparation

Soil samples were collected on April 9, 2014 from cover crop plots established on August 31, 2013. Five cores (10 cm x 3.2 cm, diameter) were taken from each plot and composited in sealed plastic bags. Soil samples were transported in a cooler and stored in a refrigerator at 2°C until they could be sieved and frozen over the next seven days. Soil samples were passed through a 2 mm sieve and thoroughly mixed. A 5 g subsample of the sieved soil was oven dried to determine soil gravimetric moisture content. Approximately 10 g of the sieved soil was weighed into 50 mL Teflon centrifuge tubes and capped. These tubes were then stored at -20°C until FAME extraction.

Lipid extraction and fractionation

The following method used to extract FAMEs was adapted from White et al. (1979). Twenty milliliters of methanolic potassium hydroxide (MeOH-KOH) were added in 10 mL increments to the centrifuge tubes containing 10 g moist soil. Tubes were vortexed after each addition and then placed in a water bath at 37°C for 1 hr with occasional shaking. After removing the tubes from the water bath, two millileters of 1N acetic acid were added to each tube to return the solutions to neutrality.

Five milliters of hexane were then added to each tube and the tubes were vortexed again. Tubes were balanced using methanol and centrifuged at 6000 rpm for 10 minutes. The resulting hexane layer at the surface of each tube was transferred to a 15 mL Pyrex tube using a pipette. This hexane extraction process was repeated with another 5 mL of hexane. Hexane extracts were then filtered through PTFE 0.2 µm syringe filters into fresh Pyrex tubes. The filtered extracts were evaporated under N_2 to small volume. Three to four drops of benzene were mixed in and extracts were evaporated until dry. Residue was then redissolved with 1 mL hexane and transferred to 2 mL amber vials. Vials were then stored in the freezer at -20°C until they could be analyzed by gas chromatography.

To prepare the samples for gas chromatography, the solvents were evaporated under N_2 until the vials were completely dry. The residues were redissolved in 500 µL hexane containing C19:0 (0.05 mg/mL) for use as an internal standard. Fifty microliter aliquots were transferred to gas chromatograph vials and capped for analysis.

Quantification and identification of FAMEs

Released FAMEs were separated on a Hewlett Packard 7890 gas chromatograph using helium as the carrier gas and a HP-Ultra 2 (Agilent) capillary column (50 m, 0.2 mm I.D., 0.33 μm film thickness). Oven temperature was held at 50˚C for 2 minutes and then increased at the rate of 40˚C·min^{-1} to a temperature of 160˚C and held for 2 minutes. Oven temperature was then increased by 3˚C·min^{-1} to 300˚C and held for 30 minutes. Injector and flame ionization detector temperatures were kept at 280˚C and 300˚C, respectively. FAMEs were identified by comparing their retention times with known standards (Bacterial Acid Methyl Esters CP Mix, Supelco USA). These identities were then confirmed with gas chromatography mass spectrometry on an Agilent 7890 gas chromatograph with a 5977 mass spectrum detector. FAME concentrations were calculated from peak areas and are reported here as nmol g^{-1} soil.

FAME nomenclature

Specific FAMEs are indicated by the total number of carbon atoms in the molecule, followed by a colon and then the number of double bonds in the molecule. If there are double bonds in the molecule, the *cis* or *trans* configuration of the bond and the position of the bonds from the carboxyl end of the molecule is indicated in parentheses. Note that some authors identify double bond position from the methyl end of the molecule rather than the carboxyl end. The prefix a- and i- indicate anteiso- or isobranching, respectively, while the prefix cy- indicates cyclopropane fatty acids, and the prefix 10Me- indicates a methyl group on the 10th carbon end from the carboxyl end of the molecule.

Soil microbial biomass and community structure

Total fatty acid content is used here as an indicator of soil microbial biomass with Zelles (1992) finding a high correlation between total fatty acid content and soil microbial biomass as measured by substrate induced respiration, phosphate content, and various enzymatic procedures.

Data analysis

To test the diversity-increased abundance hypothesis, I first characterized the effects of plant productivity on soil microbial biomass by regressing soil microbial biomass against total aboveground plant biomass using ordinary least squares regression. This was done for all the plots and just the monoculture plots. A positive linear relationship between total aboveground plant biomass and soil microbial biomass was observed for all the plots and just the monoculture plots.

Having established that total aboveground plant biomass positively affects soil microbial biomass, I tested the utility of adding an interaction term into the former model where total aboveground plant biomass interacted with either cover crop species richness or cover crop functional richness. This process controlled for the positive effects of total aboveground plant biomass on soil microbial biomass in testing for the effects of cover crop mixture diversity on soil microbial biomass.

Essentially, the diversity-increased abundance hypothesis was tested by evaluating whether increasing cover crop diversity increased soil microbial biomass on a per unit total plant biomass basis (Figure 5-1).

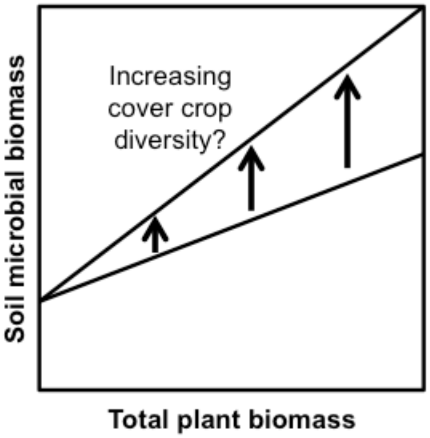

Figure 5-1. Hypothesized effect of increasing cover crop mixture diversity on soil microbial biomass.

I used the same approach to test the diversity-altered microbial community hypothesis as I did to test the diversity-increased abundance hypothesis, except the test was made on a multivariate response variable (soil microbial community structure as characterized by 18 individual FAMEs) rather than a univariate response variable (soil microbial biomass). Specifically, I first fit two models whereby total aboveground plant biomass was allowed to explain the FAME profiles. This was done first with the FAME data in absolute form—i.e., reported as nmol g^{-1}—and in relative form—i.e., reported as % total nmol. Both of these models were significant (α= 0.05) and followed up with univariate regressions for each of the individual FAMEs. Having established the effect of plant productivity on soil microbial community structure, I then tested whether adding cover crop species richness or functional richness to the model as an interaction term with total aboveground plant biomass added any additional explanation for the variance in the data. Again this was done with the FAME data both as nmol g^{-1} and % total nmol.

It should be noted that the total aboveground plant biomass values used in this analysis include a small amount of weed biomass in the form of volunteer winter wheat (a maximum of 43 g m^{-2}) in addition to cover crop biomass. All statistical analyses were conducted using R 3.1.0 (R Core Team, 2014).

RESULTS

FAMEs identified

The 18 FAMEs identified are summarized in Table 5-1 along with summary information regarding their absolute and relative quantities, as averaged across all 80 samples.

Table 5-1. Classification, names, and amounts of individual FAMEs identified in bulk soil samples—absolute and relative .

Classification		Nomenclature	FAMEs (mean±SD, N=80).	
			(nmol·g^{-1} soil)	(% total)
Saturated	Straight chain	15:0	1.5±0.3	1.46±0.08
		16:0	26±4	25.0±0.9
		17:0	1.4±0.3	1.35±0.08
	Branched chain	a15:0	8±1	7.7±0.7
		a17:0	4±0.7	4.1±0.2
		i14:0	1.3±0.2	1.2±0.1
		i15:0	12±1	11.4±0.6
		i16:0	8±1	7.2±0.3
		i17:0	3.3±0.4	3.2±0.2
		10Me18:0	3.2±0.5	3.1±0.2
		10Me19:0	4.9±0.6	4.7±0.3
	Cyclopropane	cy17:9,10	3.1±0.4	3.0±0.2
		cy19:9,10	0.9±0.2	0.9±0.1
		cy19:11,12	6.1±0.7	5.9±0.6
Unsaturated	Monounsaturated	16:1(cis11)	5.9±0.9	5.8±0.8
	Polyunsaturated	18:2(cis9,12)	13±3	12±1
		20:4	1.3±0.3	1.2±0.2
		20:5	0.5±0.1	0.5±0.1
		Total	**104±14**	-

Cover crop biomass, diversity, and soil microbial biomass

The presence of a cover crop was associated with increased total FAMEs (mean effect size = 5.6%, 95% C.I. = [2.3, 8.9%], N = 76, *p*-value = 0.001)—with total FAMEs increasing linearly with total aboveground plant biomass (Figure 5-2a). To verify that the effect observed was an effect of total aboveground plant biomass rather than mixture diversity, I further established that this effect was present when looking at just the monoculture treatments (Figure 5-2b).

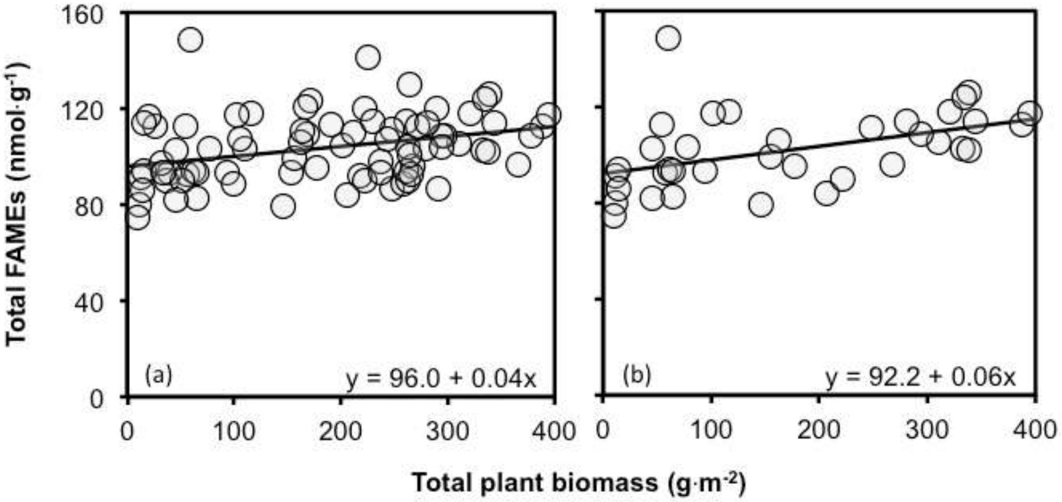

Figure 5-2. Total FAMEs extracted versus total aboveground plant biomass (N = 80) with linear regression for (a) all plots and (b) cover crop monoculture plots only.

The model for this relationship was not improved by adding cover crop species or functional richness as an interaction term with total plant biomass. In both situations the parameter estimate on the term was not significantly different from zero (Table 5-2).

Table 5-2. Parameter estimates for linear models (df = 77) relating total FAMEs to aboveground plant biomass (BIOM) with and without cover crop mixture species richness (SR) or functional richness (FR) interacting with plant biomass.

Equation	**Parameter**	**Estimate±SEM**[†]	***p*-value**
Total FAMEs ~ BIOM + BIOM:SR	Intercept	96±3****	<0.0001
	BIOM	0.038±0.017*	0.0233
	BIOM:SR	0.001±0.003^{NS}	0.7658
Total FAMEs ~ BIOM + BIOM:FR	Intercept	96±3****	<0.0001
	BIOM	0.057±0.019**	0.0050
	BIOM:FR	-0.008±0.008^{NS}	0.2775

[†] Superscripts indicate *p*-values for the following hypothesis test—H_0: parameter = 0; H_a: parameter ≠ 0. *P*-value > 0.05(^{NS}); < 0.05(*); < 0.01(**); < 0.001(***); < 0.0001(****).
Adjacent column indicates exact *p*-values.

Cover crop biomass, diversity, and soil microbial community structure

FAMEs both as nmol g^{-1} and % total nmol varied significantly as a function of total aboveground plant biomass but neither adding cover crop mixture species richness or functional richness as interaction terms with total aboveground plant biomass significantly improved the fit of these models (Table 5-3).

Follow-up univariate regressions showed the specific effects of total aboveground plant biomass on individual FAMEs (Figure 5-3). Increasing total aboveground plant biomass was associated with statistically significant increases in fifteen of the eighteen identified FAMEs (α= 0.05). The remaining three FAMEs—20:4, 20:5, and 16:1(cis11)—were not significantly affected by increases in total plant biomass, though their slope estimates were positive like the rest of the FAMEs identified.

While the concentrations of the individual FAMEs all generally increased with increasing total plant biomass, they did so at different rates. This led to some of their relative proportions being altered with increasing total plant biomass. Of the eighteen

FAMEs identified, the relative proportions of twelve were not significantly affected by increases in total plant biomass. Of the six FAMEs that were affected, three increased and three decreased in relative proportion with increasing total plant biomass.

Those that increased in relative proportion were a15:0, a17:0, and i14:0. Those that decreased in relative proportion were 16:0, i17:0, and 16:1(cis11). While 16:0 is common to many different types of organisms (Harwood and Russell, 1984), the saturated branched chain FAMEs listed here (a15:0, a17:0, i14:0, and i17:0) have been associated with bacterial organisms (Kaneda, 1991) and 16:1(cis11) has been used as an indicator of arbuscular mycorrhizal fungi (Olsson, 1999; Olsson et al., 1995).

Table 5-3. Multivariate regression results for the relationship between FAMEs as (a) nmol·g^{-1} and (b) % total nmol and aboveground plant biomass (BIOM) with and without the addition of an interaction term between plant biomass and cover crop mixture species richness (SR) and functional richness (FR).

	Equation	**Variable**	**Pillai's trace**	**F-value**	**df**[†]	***p*-value**
(a)	~ BIOM	BIOM	0.55	4.19	18, 61	<0.0001
	~ BIOM + BIOM:SR	BIOM	0.55	4.13	18, 60	<0.0001
		BIOM:SR	0.32	1.60	18, 60	0.09
	~ BIOM + BIOM:FR	BIOM	0.55	4.12	18, 60	<0.0001
		BIOM:FR	0.30	1.43	18, 60	0.15
(b)	~ BIOM	BIOM	0.55	4.53	17, 62	<0.0001
	~ BIOM + BIOM:SR	BIOM	0.55	4.45	17, 61	<0.0001
		BIOM:SR	0.28	1.36	17, 61	0.19
	~ BIOM + BIOM:FR	BIOM	0.55	4.45	17, 61	<0.0001
		BIOM:FR	0.29	1.45	17, 61	0.14

[†] Degrees of freedom (numerator df, denominator df)

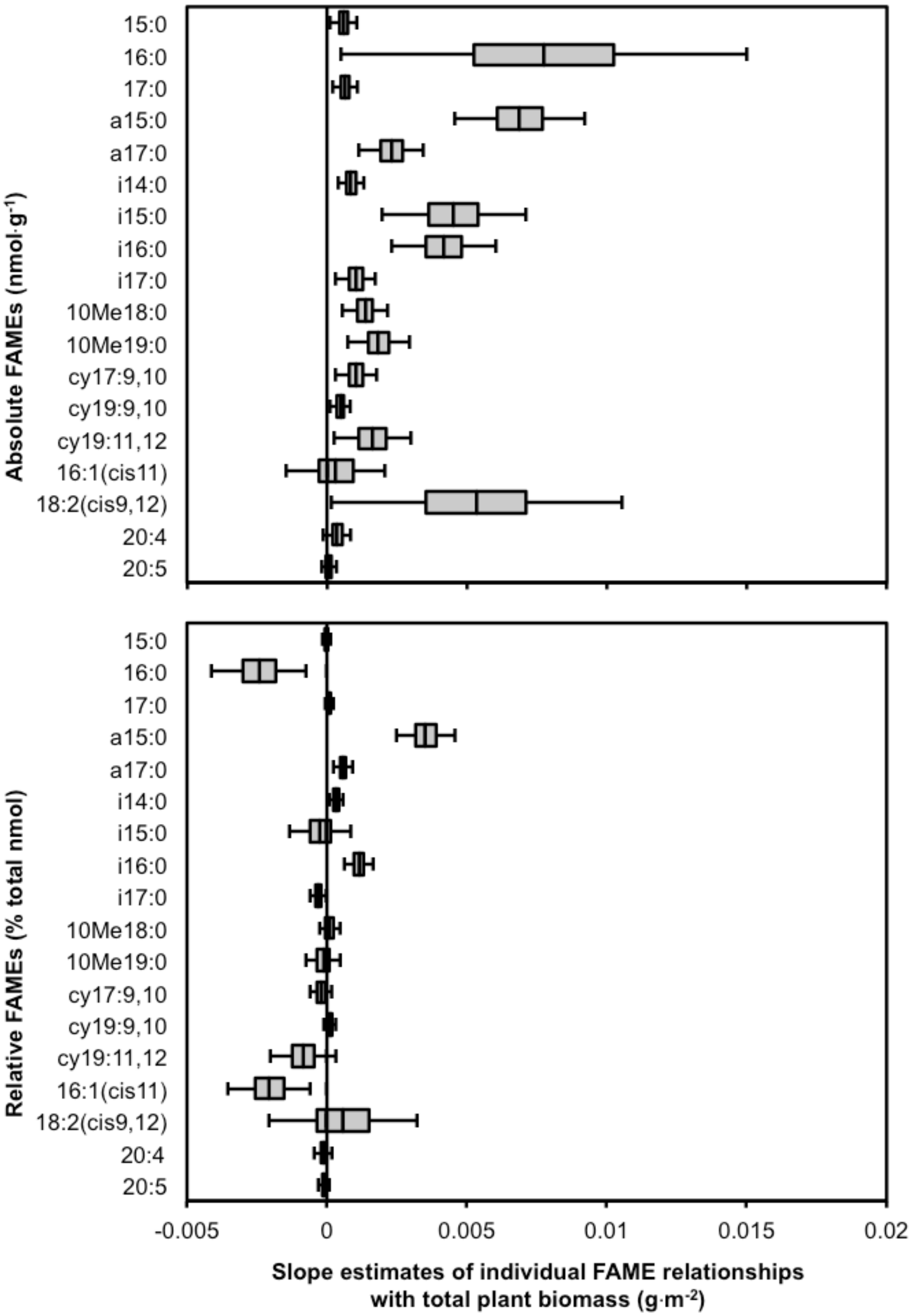

Figure 5-3. Slope estimates of relationship between FAMEs and total plant biomass. Boxes and bars represent 50% and 95% confidence intervals, respectively.

DISCUSSION

Soil microbial biomass

We observed a positive effect of cover cropping on soil microbial biomass five months after the cover crops winter killed whereas Wortman et al. (2013) observed no effect of cover cropping on soil microbial biomass one month after cover crop termination. However, between cover crop planting and soil sampling, Wortman et al. had three mechanical soil disturbance events consisting of either a disking or an undercutting of the cover crop and two interrow cultivations. In the study presented here, there were no soil disturbance events between the time of cover crop planting and the time of soil sampling. As soil disturbance can both decrease soil microbial biomass and alter soil microbial community structure (Buckley and Schmidt, 2001; Cookson et al., 2008; Doran, 1987; Drijber et al., 2000; Wortmann et al., 2008), we hypothesize that any effects of cover cropping on soil microbial biomass were masked by the three soil disturbance events in the study by Wortman et al.

Soil microbial biomass also increased with increasing plant biomass. This is consistent with observations that organic inputs tend to increase microbial biomass and withholding organic inputs tends to decrease microbial biomass (Bossio et al., 1998, Drenovsky et al., 2004; Hirsch et al., 2009; Ladd et al., 1994). While other studies have shown that the presence of vegetation increases soil microbial biomass as compared to a bare soil control, this study further shows that greater aboveground plant biomass is associated with greater soil microbial biomass.

Once variation in plant productivity was accounted for, we observed no effect of cover crop species or functional richness on soil microbial biomass. Of those studies discussed earlier that observed a positive relationship between plant mixture diversity and soil microbial biomass, all of them had a scenario in which plant productivity co-varied with plant mixture diversity. Of these studies, four of them did not address the issue (Carney and Matson, 2004; Guenay et al., 2013; Spehn et al., 2000; Stephen et al., 2000), two concluded that most of the effects of plant mixture diversity on soil microbial biomass were mediated through variations in plant biomass (De Deyn et al., 2011; Zak et al., 2003), and two of them inserted metrics of plant productivity as covariates into their models of the relationship between soil microbial biomass and plant diversity and concluded that plant mixture diversity had positive effects on soil microbial biomass beyond its effects on plant biomass (Chung et al., 2007; Eisenhauer et al., 2010),

Thus our results only appear to be in conflict with Chung et al. (2007) and Eisenhauer et al. (2010). How do we reconcile this apparent conflict? I think the answer lies in the time scale of these studies. Both of these studies took their soil samples after many years of their plots being planted to particular species richness. In the case of Chung et al. (2007), the plant mixture diversity treatments were established in 1997 and the soil samples were taken in 2003—six years later. In the case of Eisenhauer et al. (2010), the plant treatments were established in 2002 and soil samples were taken annually from 2003 to 2008 with apparent effects of planted species richness on soil microbial biomass only appearing after four years.

The narrative I find most likely here is that continued co-variation between planted species richness and plant productivity over the years led to increasing divergence in soil characteristics. As was discussed in Chapter 4, productive plots tend to promote greater soil fertility than unproductive plots in the form of greater nutrient retention and nutrient release from mineral forms. Furthermore, we would expect that over time, increased organic inputs would lead to increased soil organic matter and greater water holding capacity. Consequently, I hypothesize that this divergence in soil characteristics is then the driver for further variation in soil microbial biomass rather than planted species richness—soil microbial biomass being sensitive to both soil fertility and soil moisture (Bååth and Anderson, 2003; Doran, 1980; rev. Kennedy et al., 2004; Kennedy et al., 2005; Schimel et al., 1999; Vineela et al., 2008). A test of this would be to compare an equivalently productive monoculture and diverse mixture over time.

Soil microbial community structure

Cover cropping significantly affected soil microbial community structure. This is consistent with the findings of Carrera et al. (2007). Furthermore, increasing total aboveground plant biomass generally increased the concentrations of each individual FAMEs with some FAMEs being more affected than others. This led to alterations in soil microbial community structure as defined both by total concentrations of each FAME and relative concentrations of each FAME. Like with soil microbial biomass, however, once the effects of aboveground plant biomass were accounted for, there were no observed effects of cover crop species richness or functional richness on soil microbial community structure.

Parting thoughts regarding plant specific effects on soil microbial community characteristics

The idea that increasing plant mixture diversity should have an effect on soil microbial community characteristics is partly predicated on the idea that different plants have different effects on soil microorganisms (Wardle et al., 2004). Since different plant species are expected to vary in their litter quality, their root exudates, their effects on local soil fertility, and even their effects on the microenvironment in the form of different root and shoot architectures, it is expected that different plants should have different effects on the soil microbial community (Badri and Vivanco, 2009; Berg and Smalla, 2009; Wolfe and Klironomos, 2005).

Many studies have observed that different plant species alter soil microbial biomass and community structure in distinct ways (Bardgett et al., 1999; Batten et al., 2006; Costa et al., 2005; Fang et al., 2001; Germida et al., 1998; Grayston et al., 1998; Ibekwe and Kennedy, 1998; Innes et al., 2004; Kourtev et al., 2002, 2003; Kowalchuk et al., 2002; Kuske et al., 2002; Larkin, 2003, 2008; Larkin et al., 2010; Marschner et al., 2001, 2004; Miethling et al., 2000; Pascault et al., 2010; Ravit et al., 2003; Smalla et al., 2001; Söderberg et al., 2002; Stephen et al., 2000; Wieland et al., 2001). I wonder, however, to what degree these variations can be attributed to variations in plant productivity. Exploring this issue with data from Innes et al. (2004), I found that most of the variation in soil microbial biomass observed between plant species could be explained by variations in plant productivity (Figure 5-4).

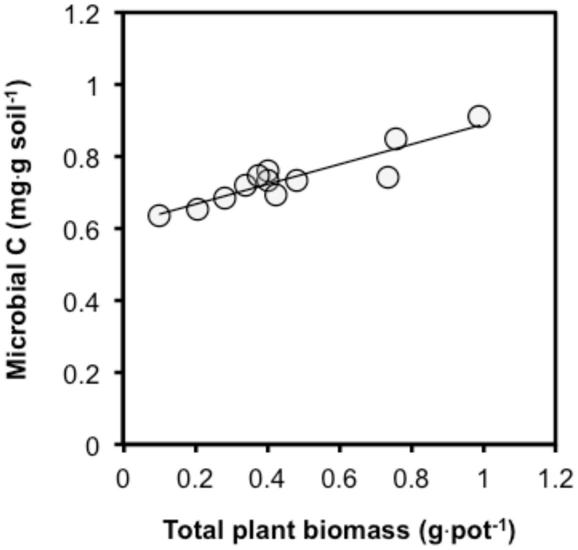

Figure 5-4. Soil microbial carbon versus total dry plant biomass (roots and shoots) for three grass species and three dicot species grown on two soils. Data from a greenhouse study by Innes et al. (2004). Linear regression: $y = 0.27x + 0.61$.

I hypothesize that this simple example extends to the more difficult to visualize situation of soil microbial community structure. Multivariate analysis may make it appear that different plants or plant mixtures of differing levels of diversity have large differing effects on soil microbial community structure, but it may be that once we account for variations in plant productivity, those differences either disappear or diminish markedly in magnitude. While it's unlikely that there is no effect of plant species on soil microbial characteristics, I propose that those effects have been previously confounded with the effects of productivity and overstated.

References

Bååth, E., and T.-H. Anderson. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol. Biochem. 35:955-963.

Badri, D. V., and J. M. Vivanco. 2009. Regulation and function of root exudates. Plant Cell Environ. 32:666–681.

Bardgett, R. D, J. L. Mawdsley, S. Edwards, P. J. Hobbs, J. S. Rodwell, and W. J. Davies. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. Funct. Ecol. 13:650-660.

Batten, K. M., K. M. Scow, K. F. Davies, and S. P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. Biol. Invasions 8:217–230.

Berg, G., and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microb. Ecol. 68:1-13.

Bernard, E. D., R. P. Larkin, S. Tavantzis, M. S. Erich, A. Alyokhin, G. Sewell, A. Lannan, and S. D. Gross. 2012. Compost, rapeseed rotation, and biocontrol agents significantly impact soil microbial communities in organic and conventional potato production systems. Appl. Soil Ecol. 52:29-41.

Bossio, D. A., K. M. Scow, N. Gunapala, and K. J. Graham. 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb. Ecol. 36:1-12.

Buckley, D. H., and T. M. Schmidt. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microb. Ecol. 42:11-21.

Buyer, J. S., J. R. Teasdale, D. P. Roberts, I. A. Zasada, and J. E. Maul. 2010. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biol. Biochem. 42:831-841.

Carney, K. M., and P. A. Matson. 2004. The Influence of Tropical Plant Diversity and Composition on Soil Microbial Communities. Microb. Ecol. 52:226-238.

Carrera, L. M., J. S. Buyer, B. Vinyard, A. A. Abdul-Baki, L. J. Sikora, and J. R. Teasdale. 2007. Effects of cover crops, compost, and manure amendments on soil microbial community structure in tomato production systems. Appl. Soil Ecol. 37:247-255.

Chapman, S. K., and G. S. Newman. 2010. Biodiversity at the plant-soil interface: microbial abundance and community structure respond to litter mixing. Oecologia 162:753-769.

Chung, H., D. Zak, P. B. Reich, and D. S. Ellsworth. 2007. Plant species richness, elevated CO2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Glob. Change Biol. 13:980-989.

Cookson, W. R., D. V. Murphy, and M. M. Roper. 2008. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. Soil Biol. Biochem.40:763-777.

Costa R., M. Gotz, N. Mrotzek, J. Lottmann, G. Berg, and K. Smalla. 2005. Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. FEMS Microbiol. Ecol. 56: 236–249.

De Deyn, G. B., H. Quirk, and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biol Lett 7:75–8.

Doran, J. W. 1980. Microbial Changes Associated with Residue Management with Reduced Tillage. Soil Sci. Soc. Am. J. 44:518-524.

Doran, J.W. 1987. Microbial biomass and mineralizable nitrogen distributions in not-illage and plowed soils. Biol. Fert.Soils 5:68-75.

Drenovsky, R. E., D. Vo, K. J. Graham, and K. M. Scow. 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb. Ecol. 48:424-430.

Drijber, R. A., J. W. Doran, A. M. Parkhurst, and D. J. Lyon. 2000. Changes in soil microbial community structure with tillage under long-term wheat-fallow management. Soil Biol. Biochem. 32:1419-1430.

Eisenhauer, N., H. Beßler, C. Engels, G. Gleizner, M. Habekost, A. Milcu, S. Partsch, A. C. W. Sabais, C. Scherber, S. Steinbeiss, A. Weigelt, W. W. Weisser, and S. Scheu. 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91(2):485-496.

Fang, C., M. Radosevich, and J. J. Fuhrmann. 2001. Characterization of rhizosphere microbial community structure in five similar grass species using FAME and BIOLOG analyses. Soil Biol. Biochem. 33:679–682.

Germida, J. J., S. D. Siciliano, J. R. de Freitas, A. M. Seib. 1998. Diversity of rootassociated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). FEMS Microbiol. Ecol. 26:43-50.

Grayston, S.J., S. Wang,, C. D. Campbell, and A. C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem. 30:369–378.

Guenay, Y., A. Ebeling, K. Steinauer, W. W. Weisser, and N. Eisenhauer. 2013. Transgressive overyielding of soil microbial biomass in grassland plant diversity gradient. Soil Biol. Biochem. 60:122-124.

Habekost, M., N. Eisenhauer, S. Scheu, S. Steinbeiss, A. Weigelt, and G. Gleixner. 2008. Seasonal changes in the soil microbial community in a grassland plant diversity gradient four years after establishment. Soil Biol.Biochem. 40:2588-2595.

Harwood, J. L., and N. Russell. 1984. Major lipid types in plants and micro-organisms. In: J. L. Harwood, and N. Russell, editors, Lipids in Plants and Microbes. Allen & Unwin, London, UK. p.7-34.

Hirsch, P. R., L. M. Gilliam, S. P. Sohi, J. K. Williams, I. M. Clark, and P. J. Murray. 2009. Starving the soil of plant inputs for 50 years reduces abundance but not diversity of soil bacterial communities. Soil Biol. Biochem. 41:2021-2024.

Ibekwe, A.M., and A.C. Kennedy. 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiol. Ecol. 26:151-163.

Innes L., P. J. Hobbs, R. D. Bardgett. 2004. The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. Biol. Fert. Soils 40:7–13.

Kaneda, T. 1991. Iso- and Anteiso-Fatty Acids in Bacteria: Biosynthesis, Function, and Taxonomic Significance. Microbiol. Rev. 55(2):288-302.

Kennedy, A.C., T.L. Stubbs, and W.F. Schillinger. 2004. Soil and Crop Management Effects on Soil Microbiology. In: F. Magdoff and R. R. Weil, editors, Soil Organic Matter in Sustainable Agriculture. CRC Press, Boca Raton, FL. p. 295-326.

Kennedy, N., J. Connolly, and N. Clipson . 2005. Impact of lime and nitrogen and plant species on fungal community structure in grassland microcosms. Environ . Microbiol. 7:780–788.

Kourtev P. S., J. G. Ehrenfeld, and M. Haggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83:3152–3166.

Kourtev P. S., J. G. Ehrenfeld, and M. Häggblom. 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biol. Biochem. 35:895–905.

Kowalchuk, G. A., D. S. Buma, W. de Boer, P. G. L. Klinkhamer, J. A. van Veen. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie van Leeuwenhoek 81:509-520.

Kuske C. R., L. O. Ticknor, M. E. Miller, J. M. Dunbar, J. A. Davis, S. M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. Appl. Environ. Microb. 68:854–1863.

Ladd J. N., M. Amato, Z. Li-Kui, and J. E. Schultz. 1994. Differential effects of rotation, plant residue and nitrogen fertilizer on microbial biomass and organic matter in the Australian alfisol. Biol. Fert. Soils 26:821–831.

Larkin, R. P. 2003. Characterization of soil microbial communities under different potato cropping systems by microbial population dynamics, substrate utilization, and fatty acid profiles. Soil Biol. Biochem. 35:1451–1466.

Larkin, R. P. 2008. Relative effects of biological amendments and crop rotations on soil microbial communities and soilborne diseases of potato. Soil Biol. Biochem. 40:1341-1351.

Larkin, R. P., T. S. Griffin, and C. W. Honeycutt. 2010. Rotation and cover crop effects on soilborne potato diseases, tuber yield, and soil microbial communities. Plant Dis. 94:1491–1502.

Lehman, R. M., T. E. Ducey, V. L. Jin, V. Acosta-Martinez, C. M. Ahlschwede, E. S. Jeske, R. A. Drijber, K. B. Cantrell, J. R. Frederick, D. M. Fink, S. L. Osborne, J. M. Novak, J. M. E. Johnson, and G. E. Varvel. 2014. Bioenerg. Res. 7:540-550.

Lehman, R. M., W. I. Taheri, S. L. Osborne, J. S. Buyer, and D. D. Douds Jr. 2012. Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. Appl. Soil Ecol. 61:300-304.

Marschner P., C. H. Yang, R. Lieberei, and D. E. Crowley. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol. Biochem. 33:1437-1445.

Marschner P., D. Crowley, and C. H. Yang. 2004. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. Plant Soil 261:199-208.

Mendes, I. C., A. K. Bandick, R. P Dick, and P. J. Bottomley. 1999. Microbial biomass and activities in soil aggregates affected by winter cover crops. Soil Sci. Soc. Am. J. 63:873-881.

Miethling, R., G. Wieland, H. Backhaus, H., and C. Tebbe. 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Microb. Ecol. 40:43-56.

Olsson, P. A., E. Bååth, I. Jakobsen, and B. Soderstrom. 1995. The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycol. Res. 99:623-629.

Olsson, P.A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. 29:303-310.

Pascault, N., L. Cécillon, O. Mathieu, C. Hénault, A. Sarr, J. Lévêque, P. Farcy, L. Ranjard, and P.-A. Maron. 2010. In situ dynamics of microbial communities during decomposition of wheat, rape, and alfalfa residues. Microb. Ecol. 60:816-828.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ravit B., J. G. Ehrenfeld, and M. M. Haggblom. 2003. A comparison of sediment microbial communities associated with *Phragmites australis* and *Spartina alterniflora* in two brackish wetlands of New Jersey. Estuaries 26:465-474.

Schimel J. P., J. M. Gulledge, J. S. Clein-Curley, J. E. Lindstrom, and J. F. Braddock. 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biol. Biochem. 31:831-838.

Schutter, M.E., and R. P. Dick. 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. Soil Sci. Soc. Am. J. 66:142-153.

Schutter, M.E., J. M. Sandeno, and R. P. Dick. 2001. Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biol. Fert. Soils 34:397-410.

Smalla K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer, and G. Berg. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl. Environ. Microb. 67:4742-4751.

Söderberg, K.H., P. A. Olsson, and E. Bååth. 2002. Structure and activity of the bacterial community in the rhizosphere of different plant species and the effect of arbuscular mycorrhizal colonisation. FEMS Microbiol. Ecol. 40:223-231.

Spehn, E. M., J. Joshi, B. Schmid, J. Alphei, and C. Kömer. 2000. Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. Plant Soil 224:217-230.

Stephan A., A. H. Meyer, and B. Schmid. 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. J. Ecol. 88:988-98.

Vineela C., S. P. Wani, Ch. Srinivasarao, B. Padmaja, and K. P. R. Vittal. 2008. Microbial properties of soils as affected by cropping and nutrient management practices in several long-term manurial experiments in the semi-arid tropics of India. Appl. Soil Ecol. 40:165-173.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629-1633.

Wardle, D.A., and K.S. Nicholson. 1996. Synergistic effects of grassland species on soil microbial biomass and activity: implications for ecosystem-level effects of enriched plant diversity. Funct. Ecol. 10:410-416.

White, D. C., W. M. Davis, J. S.Nickels, J. D. King, R. J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40:51-62.

Wieland G., R. Neumann, and H. Backhaus. 2001. Variation of microbial communities in soil, rhizosphere and rhizoplane in response to crop species, soil type, and crop development. Appl Environ. Microb. 67:5849-5854.

Wolfe B. E., and J. N. Klironomos. 2005. Breaking new ground: soil communities and exotic plant invasion. Bioscience 55:477-487.

Wortman, S., R. A. Drijber, C. A. Francis, and J. L. Lindquist. 2013. Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping system. Appl. Soil Ecol. 72:232-241.

Wortmann, C. S., J. A. Quincke, R. A. Drijber, M. Mamo, and T. Franti. 2008. Soil microbial community change and recovery after one-time tillage of continuous no-till. Agron. J. 100:1681-1686.

Zak, D. R., W. E. Holmes, D. C. White, A. D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84:2042-2050.

Zelles, L., Q. Y. Bai, T. Beck, and F. Beese. 1992. Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem. 24:317-323.

Zhang, C-B., J. Wang, W-L. Liu, S-X. Zhu, H-L. Ge, S. X. Change, J. Chang, and Y. Ge. 2010. Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland. Ecol. Eng. 36:62-68.

Chapter 6 - Cover crop mixture diversity and stability

ABSTRACT

The diversity-stability hypothesis proposes that more diverse systems are more stable than less diverse systems. The goal of this study was to evaluate the effect of increasing cover crop mixture species and functional richness on the stability of cover crop biomass production across plots within a site. Eighteen species of cover crops were used in this study representing six pre-defined functional groups—cool-season grasses, cool-season legumes, cool-season brassicas, warm-season grasses, warm-season legumes, and warm-season broadleaves. Up to forty treatments reflecting varying levels of species richness (1, 3, 6, 9, and 18 species) and functional richness (1, 2, 3, and 6 functional groups) were grown at seven sites across southeastern Nebraska. All species were equally represented at each level of diversity. Cover crop planting dates ranged from July 20 to September 19. Species specific aboveground plant biomass measurements were taken prior to winterkill, ranging from 50 to 84 days after cover crop planting. The standard deviations of each treatment at each site were regressed against the mean productivities of each treatment at each site. The diversity-stability hypothesis was tested by evaluating whether increasing species or functional richness decreased the slopes of these regression lines. That is, the hypothesis was tested by evaluating whether the standard deviations were less for more diverse treatments than for less diverse treatments accounting for variation in biomass productivity. Increasing species and functional richness had weakly negative but non-significant effects on the slope of the regression in both situations. Thus, there was minimal evidence that increasing cover crop mixture diversity stabilized aboveground biomass productivity across fields.

INTRODUCTION

The diversity-stability hypothesis asserts that more diverse systems are more stable systems. While there is considerable debate regarding this hypothesis in the field of ecology (rev. McCann, 2000), the idea has been essentially imported into the field of agriculture as a proven principle (e.g., Malézieux et al., 2009). For example, it's conventional wisdom in agriculture that plant mixtures are more stable than monocultures (e.g., Anil et al., 1998; Horwith, 1985). The idea is that if a single crop fails, another crop may be able to compensate for it in a mixture (Griffin et al., 2009; Willey, 1979). While this makes intuitive sense there is actually little empirical evidence to favor this assertion (Liebman, 1995; Trenbath, 1974; Vandermeer and Schultz, 1990; Willey et al., 1983). The goal of this study was to determine whether or not increasing cover crop mixture diversity increases the stability of cover crop biomass productivity.

The term "stability" is used in the ecological and agricultural literature to refer to many different ideas, which is part of the reason for the controversy around the diversity-stability hypothesis (Ives and Carpenter, 2007). For example, the term "stability" has been variously used to refer to consistency of community composition, resistance or resilience to disturbance, and decreased temporal or spatial variability in response to variable abiotic conditions (Hooper et al., 2005). In this study, I define increased stability as decreased variation in biomass performance across variable environmental conditions.

Coefficient of variation, the ratio of the standard deviation to the mean, is the single most widely used indicator of stability both in the fields of ecology and agriculture, with a low coefficient of variation considered to be an indicator of high stability. In the literature, a distinction is often made between temporal stability, which refers to a low coefficient of variation across time, and spatial stability, which refers to a low coefficient of variation across quadrats within a plot, across plots within a site, or across sites within a region. While most studies focus on temporal stability (Gross et al., 2014; Jiang and Pu, 2009), and this study focuses on spatial stability, I would like to suggest that these are not so much discrete categories as they are variants on the same theme. That is, both temporal and spatial stability represent consistency of performance in the face of environmental variation. It is simply the scale and type of environmental variation that varies between typical temporal and spatial measurements.

Despite coefficient of variation being the traditional metric for stability, I find there to be one major issue with this approach, and that is that the coefficient of variation of productivity tends to be elevated at low levels of productivity as compared to high levels of productivity. Consequently, in studies where diversity co-varies with productivity, positive diversity-stability relationships may simply be the result of the relationship between productivity and stability. Thus, another goal of this study is to address the issue of the covariance of diversity with productivity covariance in our understanding of diversity-stability patterns.

MATERIALS AND METHODS

Research sites, experimental design, and data collection

Seven sites across southeastern Nebraska were planted with twenty to forty cover crop treatments reflecting varying levels of species and functional richness. Details regarding the location of these sites, the composition and establishment of these treatments, and the sampling of these treatments can be found in Chapter 2.

Data analysis

The standard metric used to evaluate stability is the coefficient of variation (C_v) of stand biomass, which is calculated as the standard deviation (σ) divided by the mean (μ) biomass, or more accurately, it is estimated as the sample standard deviation (s) divided by the sample mean ($\overline{x}$) biomass. In the results that follow, this ratio will be further multiplied by 100 and expressed as a percentage.

$$C_v = \sigma/\mu * 100 \text{ (estimated as } \hat{C}_v = s/\overline{x} * 100)$$

In diversity-stability studies, the most common approach to evaluating the effect of diversity on stability is to regress estimated coefficients of variation against a diversity metric—most often species richness (e.g., Biondini, 2007; Hector et al., 2010; McNaughton, 1977; McNaughton, 1993; Pfisterer et al., 2004; Tilman, 1996; van Ruijven and Berendse, 2007). I have avoided this approach because diversity co-varies with biomass productivity and coefficients of variation are sensitive to biomass productivity. Consequently, the results of simply regressing coefficients of variation against diversity can be misleading because the effects of diversity on stability are confounded with the effects of biomass productivity on stability.

Coefficient of variation is calculated by dividing the standard deviation of a treatment by its mean biomass productivity, so it would seem that the productivity effects are inherently accounted for in coefficient of variation calculations. The issue is not with the mean itself but rather with the interaction of the mean and the standard deviation. Coefficients of variation were relatively constant beyond a certain level of mean biomass. At low levels of mean biomass, however, coefficients of variation became unstable, which meant that less productive treatments on average had higher coefficients of variation than more productive treatments.

To avoid mistaking the effects of biomass productivity on stability with the effects of diversity on stability in testing the diversity-stability hypothesis, I regressed standard deviations for each treatment at each site against mean cover crop biomass for each treatment at each site and then tested whether increasing cover crop diversity—as measured by cover crop mixture species and functional richness—decreased the slope of this relationship (Figure 6-1).

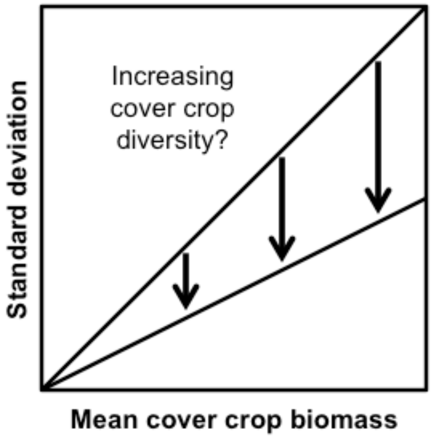

Figure 6-1. Hypothesized effect of increasing cover crop diversity on the relationship between standard deviation and mean cover crop biomass for each treatment.

Tilman et al. (2006) used a similar approach to look at the stability of treatments across time as a way of looking at temporal stability. Here, however, we looked at the stability of treatments across plots within sites as a way of evaluating spatial stability. All statistical analyses were conducted using R 3.1.0 (R Core Team, 2014).

RESULTS

Correlation between coefficient of variation and cover crop mixture diversity

To show how simply plotting coefficients of variation against diversity metrics might be misleading, I have done so with the data here (Figure 6-2). Coefficient of variation does decrease with increasing species and functional richness, but that's not to say that increasing species and functional richness increases stability.

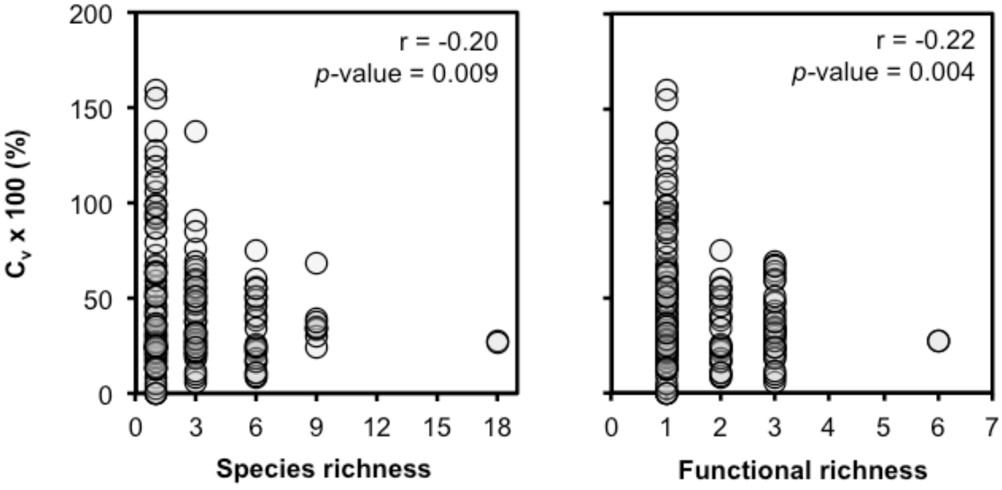

Figure 6-2. Coefficient of variation for each treatment at each site plotted by species richness (left) and functional richness (right). Pearson correlation coefficients (r) given with *p*-values for the following test—H_0: r = 0; H_a: r ≠ 0.

Relationship between mean aboveground biomass and coefficient of variation

If we look at the relationship between coefficient of variation and mean cover crop biomass, we find that at low biomass, coefficient of variation tends to be greater and less consistent than at larger biomass (

Figure 6-3).

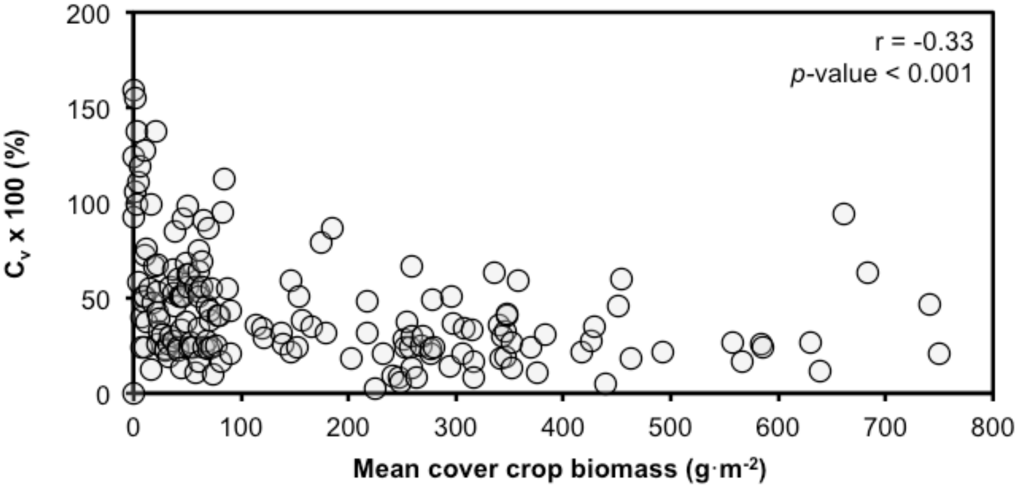

Figure 6-3. Coefficient of variation for each treatment at each site plotted by mean cover crop biomass. Pearson correlation coefficients (r) also given with *p*-values for the following test—H_0: r = 0; H_a: r ≠ 0.

I propose that this is because small amounts of experimental error at high levels of mean biomass have marginal effects on coefficient of variation due to large denominator values while at low levels of mean biomass, small amounts of error amplify into dramatic effects on coefficient of variation due to small denominator values. Thus, the pattern that we observed in Figure 6-2 could simply have been due to the fact that low diversity treatments tended to have less biomass in our study and treatments with less biomass tend to have higher coefficients of variation.

Effect of diversity on stability

Thus, to evaluate the effect of diversity on stability, the effect of biomass on stability needed to be accounted for. I did this by first modeling the relationship between standard deviation and mean cover crop aboveground biomass (Figure 6-4). As mean cover crop biomass went up, so did the standard deviation.

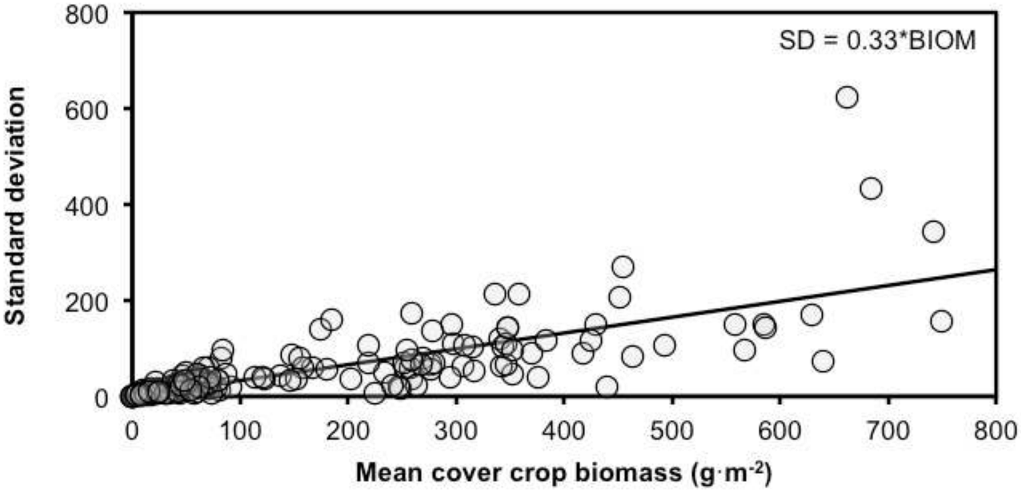

Figure 6-4. Standard deviation versus mean cover crop aboveground biomass for each treatment as averaged across plots within each site. Line represents ordinary least squares regression with intercept term removed.

Then I evaluated whether the slope of this relationship was affected by cover crop mixture diversity. I did this by evaluating the utility of adding an interaction term between cover crop aboveground biomass and species richness as well as between cover crop aboveground biomass and functional richness. The results of this analysis are summarized in Table 6-1. While each of the interaction term parameter estimates was marginally negative, in no instance was the interaction term parameter estimate different from zero (α=0.05).

Table 6-1. Parameter estimates, degrees of freedom, and *p*-values for linear models relating standard deviation (SD) to mean cover crop aboveground biomass (BIOM) with and without cover crop species richness (SR) and functional richness (FR) interacting with cover crop aboveground biomass.

Equation[†]	**df**	**Parameter**[‡]	**Estimate±SEM**[§]	***p*-value**
SD ~ BIOM (Base model)	172	BIOM	$0.33 \pm 0.02^{****}$	<0.0001
SD ~ BIOM + BIOM:SR	171	BIOM	$0.35 \pm 0.02^{****}$	<0.0001
		BIOM:SR	-0.006 ± 0.005^{NS}	0.23
SD ~ BIOM + BIOM:FR	171	BIOM	$0.38 \pm 0.03^{****}$	<0.0001
		BIOM:FR	-0.03 ± 0.01^{NS}	0.07

[†] Standard deviations and mean biomass determined for each treatment across plots within each site.
[‡] Intercepts fixed to zero.
[§] Superscripts indicate *p*-values for the following hypothesis test—H_0: slope = 0; H_a: slope ≠ 0.
P-value > $0.05(^{NS})$; < $0.05(^{*})$; < $0.01(^{**})$; < $0.001(^{***})$; < $0.0001(^{****})$.

DISCUSSION

Increased species and functional richness was certainly correlated with increased stability as measured by decreased coefficients of variation. However, I assert that most of this effect was mediated by the covariance of diversity with productivity. Once the effect of productivity was accounted for, there was only a marginal effect of species and functional richness on stability. I suspect that if we were to revisit past studies evaluating the effect of plant mixture diversity on stability, we would find that much of the variation in stability is mediated by variations in biomass rather than diversity.

For example, Karpenstein-Machan et al. (2000) and Rao and Willey (1980) concluded that intercrops are more stable than sole crops because their coefficients of variation were lower than those of the sole crops, but in both studies, the intercrops were also more productive than the sole crops. Contrast that, however, with the work of Smith et al. (2014) and Wortman et al. (2012) in cover crop mixtures, where the mixtures were not necessarily the most productive or the most stable as measured by coefficients of variation. Then consider Figure 6-5, which shows the relationship between coefficient of variation and mean crop biomass for an intercropping study by Szumigalski and Van Acker (2005). Over 80% of the variation in coefficient of variation can be explained by mean crop biomass. Coefficient of variation is clearly sensitive to mean biomass, and yet the effects of biomass on stability are so rarely addressed in diversity-stability studies.

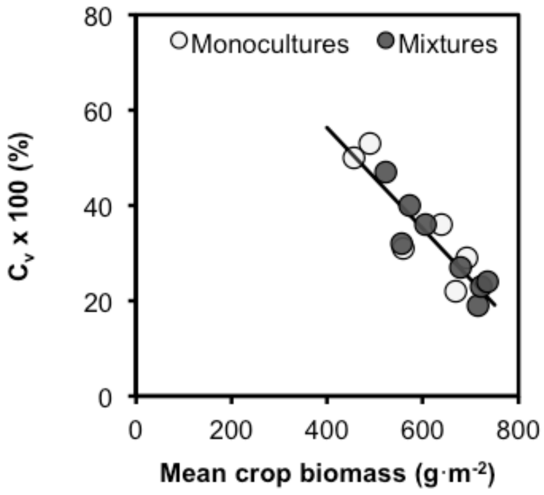

Figure 6-5. Coefficient of variation versus mean dry crop biomass for wheat, pea, and canola in monoculture and in mixtures. Data is from Szumigalski and Van Acker (2005). Data is aggregated across two sites—Kelburn and Carmen, Manitoba—and three years—2001-2003. Linear regression: $y = 98.9 – 0.1x$; $r^2 = 0.81$.

In an effort to resolve the debate around the diversity-stability hypothesis, multiple groups have conducted meta-analyses on the existing literature (Balvanera et al., 2006; Campbell et al., 2001; Cardinale et al., 2013; Gross et al., 2014; Jiang and Pu, 2009). These meta-analyses have concluded that increasing diversity has a positive effect on stability. However, while it's true that many studies have shown that increasing species richness is correlated with decreased coefficients of variation, this does not necessarily lead to the conclusion that increased species richness causes increased stability. In most of the original studies referenced by the meta-analyses, diversity co-varies with productivity and little is done in the original studies or the meta-analyses to account for the effect of productivity on coefficients of variation.

We find that in this study and others that once we control for the effect of biomass on stability, there appears to be minimal effect of diversity on stability. For the purposes of cover crop management, we found little evidence that increasing cover crop mixture diversity increased field-scale stability.

Parting thoughts regarding diversity-stability effect size expectations

The diversity-stability hypothesis is predicated on the idea that different species thrive and fail under different conditions and that the presence of many species insures that at least some species will thrive under variable environmental conditions. One of the ideas regarding why cover crop mixtures should be used is based on a similar logic: that by having many species, we increase the likelihood that at least one species will establish successfully. These are reasonable assumptions, and so I want to address what I see as the reasons for this study not observing a greater effect of diversity on stability: (1) limited species differentiation and (2) low environmental heterogeneity.

While the 18 species used in this study were all quite different from a cover cropping perspective—representing the wide range of cover crops used in the region—they weren’t actually very different from a broader botanical perspective. They all thrived in roughly the same conditions, and if the conditions were unfavorable—too dry, too cold, too shaded—all 18 species failed together. Consequently, while the 18-species mixture might be species rich, it wasn’t all that diverse in terms of species differentiation, and it’s not surprising then that its performance was no more stable than the monocultures. So I think one of the reasons for a minimal effect of diversity on stability in this study was the low amount of differentiation among species.

The other reason I think we didn’t observe much of an effect of diversity on stability was that the conditions across a single agricultural field are typically not that variable. The climatic and soil conditions were highly consistent within field at a given locations. Consequently, there was no reason to think that a species might fail in some parts of the field but thrive in other parts of the field, which is the expectation driving the diversity-stability hypothesis.

I conclude that we might expect more of an effect of diversity on stability in situations with high species differentiation and high environmental heterogeneity.

References

Anil, L., J. Park, R. H. Phipps, and F. A. Miller. 1998. Temperate intercropping of cereals for forage: A review of the potential for growth and utilization with particular reference to the UK. Grass Forage Sci. 53:301-317.

Balvanera, P., A. B. Pfisterer, N. Buchmann, J. He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9(10):1146-1156.

Biondini, M. 2007. Plant diversity, production, stability, and susceptibility to invasion in restored northern tall grass prairies (United States). Restor. Eco. 15:77-87.

Campbell, V., G. Murphy, and T. N. Romanuk. 2011. Experimental design and the outcome and interpretation of diversity-stability relations. Oikos 120:399-408.

Cardinale, B. J. K. Gross, K. Fritschie, P. Flombaum, J. W. Fox, C. Rixen, J. van Ruijven, P. B. Reich, M. Scherer-Lorenzen, and B. J. Wilsey. 2013. Biodiversity simultaneously enhances the production and stability of community biomass, but the effects are independent. Ecology 94:1697-1707.

Griffin, J. N., E. J. O'Gorman, M. C. Emmerson, S. R. Jenkins, A. M. Klein, M. Loreau, and A. Symstad. 2009. Biodiversity and the stability of ecosystem functioning. In: S. Naeem, D. E. Bunker, A. Hector, M. Loreau, and C. Perrins, editors, Biodiversity, ecosystem functioning, and human wellbeing: an ecological and economic perspective. Oxford University Press, Oxford, UK. p. 78-93.

Gross, K., B. J. Cardinale, J. W. Fox, A. Gonzalez, M. Loreau, H. Wayne Polley, P. B. Reich, and J. van Ruijven. 2014. Species Richness and the Temporal stability of Biomass Production: A New Analysis of Recent Biodiversity Experiments. The American Naturalist 183(1):1-12.

Hector, A., Y. Hautier, P. Saner, L. Wacker, R. Bagchi, J. Joshi, M. Scherer-Lorenzen, E. M. Spech, E. Bazeley-White, M. Weilenmann, M. C. Caldeira, P. G. Dimitrakopoulos, J. A. Finn, K. Huss-Danell, A. Jumpponen, C. P. H. Mulder, C. Palmborg, J. S. Pereira, A. S. D. Siamantxiouras, A. C. Terry, A. Y. Troumbis, B. Schmid, and M. Loreau. 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. Ecology 91:2213-2220.

Hooper, D. U., F. S. Chapin, J. J. Ewell, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. 75:3-35.

Horwith, B. 1985. A Role for Intercropping in Modern Agriculture. BioScience 35(5):286-291.

Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. Science 317:58-62.

Jiang, L., and Z. C. Pu. 2009. Different effects of species diversity on temporal stability in single-trophic and multitrophic communities. Am. Naturalist 174:651-659.

Karpenstein-Machan, M., and R. Stuelpnagel. 2000. Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on a subsequent maize crop. Plant Soil 218:215-232.

Liebman, M. 1995. Polyculture cropping systems. In: M. Altieri, editor, Agroecology: The science of sustainable agriculture. 2nd ed. Westview Press, Boulder, CO. p. 205-218.

Malézieux, E., Y. Crozat, C. Dupraz, M Laurans, D. Makowski, H. Oxier-Lafontaine, B. Rapidel, S. de Tourdonnet, and M. Valantin-Morison. 2009. Mixing plant species in cropping systems: concepts, tools and models – a review. Agron. Sustain. Dev. 29:43-62.

McCann, K. S. 2000. The diversity-stability debate. Nature 405:228-233.

McNaughton, S. J. 1977. Diversity and Stability of Ecological Communities: A Comment on the Role of Empiricism in Ecology. Am. Naturalist 111(979):515-525.

McNaughton, S. J. 1993. Biodiversity and function of grazing ecosystems. In: E-D., Schulze, and H. A. Mooney, editors, Biodiversity and Ecosystem Function. Springer-Verlag, Berlin, Germany. p. 361-383

Pfisterer, A. B., J. Joshi, B. Schmid, and M. Fischer. 2004. Rapid decay of diversity-productivity relationships after invasion of experimental plant communities. Basic Appl. Ecol. 5:5-14.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rao, M. R., and R. W. Willey. 1980. Evaluation of Yield Stability in Intercropping: Studies on Sorghum/Pigeonpea. Exp. Agric. 16(2):105-116.

Smith, R. G., L. W. Atwood, and N. D. Warren. 2014. Increased Productivity of a Cover Crop Mixture is Not Associated with Enhanced Agroecosystem Services. PLoS ONE 9(5): e97351. doi:10.1371/journal.pone.0097351

Szumigalski, A., and R. Van Acker. 2005. Weed Suppression and Crop Production in Annual Intercrops. Weed Science 53(6):813-825.

Tilman, D. 1996. Biodiversity: population versus ecosystem stability. Ecology 77:350-363.

Tilman, D. P. B. Reich, and J. M. H. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441:629-632.

Trenbath, B. R. 1974. Biomass productivity of mixtures. Adv. Agron. 26:177-210.

van Ruijven, J., and F. Berendse. 2007. Contrasting effects of diversity on the temporal stability of plant populations. Oikos 116:1323-1330.

Vandermeer, J. H., and B. Schultz. 1990. Variability, stability and risk in intercropping: some theoretical considerations. In: S.R. Gliessman, editor, Agroecology: researching the ecological basis for sustainable agriculture. Springer, Berlin, Germany. p. 205-232.

Willey, R. W. 1979. A scientific approach to intercropping research. In: R. W. Willey, editor, Proceedings of the International Workshop on Intercropping. 10-13 Jan. 1979. Hyderabad, India. p. 4-14.

Willey, R. W., M. Natarajan, M. S. Reddy, M. R. Rao, P. T. C. Nambiar, J. Kannaiyan, and V. S. Bhatnagar. 1983. Intercropping studies with annual crops. In: J. Nugent, and M. O'Connor, editors, Better Crops for Food. Ciba Foundation Symposium 97. Pitman, London, UK. p. 83-99.

Wortman, S. E., C. A. Francis, and J. L. Lindquist. 2012. Cover crop mixtures for the Western Corn Belt: opportunities for increased productivity and stability. Agron. J. 104:699-705.

Chapter 7 - Conclusions

I want to take this last chapter as an opportunity to summarize the findings of this study, to apply these findings to cover crop management, and to reflect a little on the large and growing body of diversity research in both the ecological and agricultural sciences.

In Chapter 2, I demonstrated that increasing cover crop mixture diversity does increase average productivity. However, I argued that there was no need to invoke niche complementarity or increased resource use efficiency to explain this result. Rather, I explained the observation as the simple result of low yielding species pulling down the average production of the monocultures. In Chapters 3, 4, 5, and 6 I discussed how diversity is often correlated to metrics of weed suppression, soil nutrient retention, soil microbial biomass, soil microbial community structure, and stability, but also that these correlations can largely be explained by variations in productivity. I drew from not only my research, but pulled out the research of past workers to demonstrate this point.

If our concern is increasing weed suppression, nutrient retention, soil microbial biomass, or stability of biomass productivity, then we should focus our attention on increasing cover crop biomass rather than cover crop mixture diversity. Productive monocultures were found to be just as good at suppressing weeds, retaining nutrients, increasing soil microbial biomass, and performing stably across variable environments as productive mixtures. We found no evidence that increasing cover crop mixture diversity enhances any of these functions.

The overwhelming pattern in diversity research is to manipulate diversity, measure a function, relate the two metrics and conclude causation—that is, to conclude that diversity affects the function. This violates one of the core principles of science, which is that correlation does not imply causation, and yet this approach is ubiquitous in the field of diversity research. The point that I re-iterate throughout this dissertation is that in most diversity studies diversity co-varies with biomass productivity, and biomass productivity has substantial effects on function, which may drive most, if not all, of the apparent effects of diversity on function.

It’s difficult to imagine a scenario in which we can ever control for all the co-varying and confounding factors in diversity research. By it’s very nature, when we compare diverse plant assemblages to monocultures we allow many different variables to vary at once. Nevertheless, it’s not until we at the least control for biomass productivity that we can start to guess at the true magnitude of diversity effects on function.