

# Managing cover crop mixtures over a decade via species replacement and seeding rate adjustment

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## RESEARCH LETTER

# Managing cover crop mixtures over a decade via species replacement and seeding rate adjustment

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## Abstract

Cover crop mixtures provide ecosystem services, but species' relative abundance in mixtures is challenging to manage. We report on an 11-year experiment where our main objective was to use species selection and seeding rate adjustments over time to increase the evenness of mixtures. Replacing rye with triticale and red clover with crimson clover while adjusting seeding rates resulted in mixtures that were more even and closer to the desired composition (greater legume biomass) than the original communities. For example, the first version of a six-species mixture produced biomass composed of 81% grass, 5% brassica, and 14% legume, but after adjustments, subsequent versions contained 25% grass, 10% brassica, and 65% legume biomass. Substituting a less aggressive grass for a dominant grass and a more aggressive legume for a weaker legume better balanced the mixture to meet farmers' ecosystem service goals, as did reducing the proportion of grass seed in the mixtures.

**Abbreviations:** NMDS, non-metric multidimensional scaling; PERMANOVA, permutational analysis of variance.

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### Plain Language Summary

Crops planted on fields when the main crops are not growing are known as cover crops, which are becoming more popular because of their potential to provide benefits to fields like improving soil health and reducing weeds. However, cover crops grow differently based on choices farmers make, including amount of seed planted and which species are planted. We investigated how changing the amount of seed and the species planted affected the growth of cover crops. We planted six species in three mixtures over 11 years. After the first 3 years, we updated the species and seed amounts based on farmer feedback and our study goals, which were not being met with the original design. The changes we made allowed the cover crops in our mixtures to grow more evenly and not outcompete one another. Our results show that it is possible to affect the way multiple species of cover crops grow together by changing the identity of the species in the mixture and changing the amount of seed planted.

## 1 | INTRODUCTION

Cover crop adoption is increasing, along with a specific interest in multi-species cover crop mixtures (SARE/CTIC/ASTA, 2023). Mixtures may provide multiple ecosystem services and can minimize disservices and tradeoffs of monocultures (Finney et al., 2017). However, planting mixtures does not automatically convey greater benefits than monocultures, as many factors affect the “expression” of a mixture (the actual plant community, measured as both species identity and proportions).

Designing reliable cover crop mixtures is challenging because expression varies with species identity and planting density (McKenzie-Gopsill et al., 2022), seeding depth (Müller et al., 2017), soil nutrient levels (Baraibar et al., 2020), and climatic conditions (Baraibar et al., 2020; Boniface et al., 2024). Further, a seeded mixture may not express evenly if one species dominates, which has been found with cereal rye (*Secale cereale*; Boniface et al., 2024; Murrell et al., 2017) and canola (*Brassica napus*; White et al., 2017). If a farmer plants a mixture for a specific purpose, such as providing nitrogen (SARE/CTIC/ASTA, 2023), but the legume is outcompeted, then the mixture has not met the farmer’s goals. Prior research has explored fine-tuning of mixtures by planting them across many farms distributed in space (Baraibar et al., 2020; Boniface et al., 2024). Yet, for an individual farmer, fine-tuning requires iterative adjustments within the same fields, which is the subject of this paper.

In 2012, we established a long-term cover crop diversity experiment to observe the species interactions and ecosystem service provisioning of specific cover crop mixtures. We co-designed the original treatments and modifications over time (implemented as different study versions that we designate V1, V2, and V3) with an advisory board of 5–10 farmers

drawing on structured co-learning activities (LaChance et al., 2015), on-farm research in which we planted cover crop mixtures on advisors’ farms (Baraibar et al., 2018; White et al., 2017), and discussions at annual advisory board meetings. We made advised adjustments to improve cover crop mixture design with the goal of increasing mixture evenness and legume biomass within mixtures. Herein, our objective is to evaluate the 11-year dataset to determine whether our temporal changes in species selection and seeding rates (three study versions) have increased mixture evenness by decreasing grass dominance and increasing legume productivity. Our results begin to fill a knowledge gap on seeding rate and species selection in a long-term cover crop mixture experiment, which are cornerstone approaches in agronomy, but which have rarely been applied in field experiments, especially with more than two species in mixtures.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

The original study (Version 1, V1 herein) was initiated in central Pennsylvania in 2012. Six cover crop species were planted in monocultures and three mixtures (Table 1): the three-species mixture (3Spp), the four-species and five-species mixtures (combined as 4Spp), and the six-species mixture (6Spp). Cover crops were planted during the window between wheat (*Triticum aestivum*) and corn (*Zea mays*) in a corn–soybean (*Glycine max*)–wheat rotation in a randomized complete block full-entry design (Murrell et al., 2017; Supporting Information).

In V1, cereal rye was found to mature quickly, outcompete other cover crop species (Murrell et al., 2017), and have a high

C:N ratio, which decreased nitrogen availability to the following cash crop (Finney et al., 2017). Thus, for Version 2 (V2, first planted in 2015), we replaced cereal rye with triticale (*× Triticosecale*), a wheat-rye hybrid expected to not outcompete other species because it is shorter and matures slower than cereal rye (Salmon et al., 2004). Crimson clover (*Trifolium incarnatum*) replaced red clover in the 3Spp and 6Spp mixtures in V2 and was added to the 4Spp mix to make it a five-species mixture. We expected crimson clover to (1) better establish from a deeper seeding depth because of its larger seed (a cone seeder was used to plant all cover crops at one uniform depth) and (2) increase biomass production and nitrogen provisioning compared to red clover when planted as a winter annual (Blesh et al., 2019). Red clover was eliminated from the species list in Version 3 (V3, first planted in 2019) due to its chronic underperformance. We also reduced seeding rates (Table 1) from “over-seeding” to the equivalent of 100% of the monoculture rates.

## 2.2 | Data collection

Cover crop communities were quantified via stand counts (2–3 weeks after seeding), fall biomass (before a killing frost), and spring biomass (immediately prior to cover crop termination). Three sub-samples were collected in each plot using a 50 cm × 50 cm quadrat as described in Murrell et al.

### Core Ideas

- Cereal rye aggressively dominated cover crop mixture communities.
- Species replacement and seed rate adjustment reduced grass domination and improved clover biomass production.
- Fine-tuning to achieve desired cover crop mixture expression is possible, despite year and environment effects.

(2017). Here, data for cereal rye and triticale were re-labeled “GRASS” as both species filled the role of winter-hardy grass in our functional group study design. Similarly, data for red clover and crimson clover (as well as the combination of the two species in the V2 five-species mixture) were re-labeled “CLOVER.”

## 2.3 | Statistical analyses

To determine if species and seeding rate changes affected mixture expression, we ran permutational analysis of variance (PERMANOVA) tests using the *adonis2* function from the *vegan* package in R (Oksanen et al., 2024). Strip, a variable

TABLE 1 Cover crop mixture composition for each of the three versions of the study.

|               | “CLOVER”                                          | Pea                                       | “GRASS”                                      | Oat                                       | Canola                                      | Radish                                       | Total seeding rate |
|---------------|---------------------------------------------------|-------------------------------------------|----------------------------------------------|-------------------------------------------|---------------------------------------------|----------------------------------------------|--------------------|
| Version 1     | <i>Trifolium pratense</i><br>600/m <sup>2</sup>   | <i>Pisum sativum</i><br>60/m <sup>2</sup> | <i>Secale cereale</i><br>500/m <sup>2</sup>  | <i>Avena sativa</i><br>300/m <sup>2</sup> | <i>Brassica napus</i><br>400/m <sup>2</sup> | <i>Raphanus sativus</i><br>60/m <sup>2</sup> |                    |
| Three-species | 0.50                                              | 0.50                                      | 0.20                                         | —                                         | —                                           | —                                            | 1.20               |
| Four-species  | 0.50                                              | 0.50                                      | 0.20                                         | —                                         | 0.50                                        | —                                            | 1.70               |
| Six-species   | 0.25                                              | 0.25                                      | 0.20                                         | 0.25                                      | 0.25                                        | 0.33                                         | 1.50               |
| Version 2     | <i>Trifolium</i> spp.<br>600/m <sup>2</sup>       | <i>Pisum sativum</i><br>60/m <sup>2</sup> | <i>× Triticosecale</i><br>300/m <sup>2</sup> | <i>Avena sativa</i><br>300/m <sup>2</sup> | <i>Brassica napus</i><br>400/m <sup>2</sup> | <i>Raphanus sativus</i><br>60/m <sup>2</sup> |                    |
| Three-species | 0.39 <sup>a</sup>                                 | 0.39                                      | 0.23                                         | —                                         | —                                           | —                                            | 1.01               |
| Five-species  | 0.44 <sup>b</sup>                                 | 0.22                                      | 0.19                                         | —                                         | 0.15                                        | —                                            | 1.00               |
| Six-species   | 0.33 <sup>a</sup>                                 | 0.33                                      | 0.09                                         | 0.09                                      | 0.07                                        | 0.09                                         | 1.00               |
| Version 3     | <i>Trifolium incarnatum</i><br>600/m <sup>2</sup> | <i>Pisum sativum</i><br>60/m <sup>2</sup> | <i>× Triticosecale</i><br>300/m <sup>2</sup> | <i>Avena sativa</i><br>300/m <sup>2</sup> | <i>Brassica napus</i><br>400/m <sup>2</sup> | <i>Raphanus sativus</i><br>60/m <sup>2</sup> |                    |
| Three-species | 0.39                                              | 0.39                                      | 0.23                                         | —                                         | —                                           | —                                            | 1.01               |
| Four-species  | 0.33                                              | 0.33                                      | 0.19                                         | —                                         | 0.15                                        | —                                            | 1.00               |
| Six-species   | 0.33                                              | 0.33                                      | 0.09                                         | 0.09                                      | 0.07                                        | 0.09                                         | 1.00               |

Note: Species are organized by functional groups with monoculture planting rates (live seed per m<sup>2</sup>) immediately beneath each species name. Monoculture rates were chosen to meet the needs of our farmer advisory panel with help from regional experts and literature. Decimal values indicate the proportion of that species' monoculture that was used to calculate the seeding rate for each species present in each mixture. GRASS refers to the winter-hardy grass species used, and CLOVER refers to the winter-hardy legume species used.

<sup>a</sup>*Trifolium incarnatum* seed only.

<sup>b</sup>*Trifolium pratense* seed at 0.22 plus *Trifolium Incarnatum* seed at 0.22.

representing both field block and study year, was defined as the “block” within the permutational design, and we ran the standard 999 permutations for each test. Study version (V1, V2, or V3) and the interaction between version and study year (with only one version being planted in any single year) were predictor variables, and the matrices of species’ percentages of the community biomass for fall and spring for each mixture were response variables. Altogether, we ran six separate PERMANOVA tests in R version 4.4.0 (R Core Team, 2024). While we acknowledge that within the study design no two versions were ever directly compared in the same year, we feel our analyses and interpretation of the version effects are robust because (1) we have data for multiple years for each version and (2) we found no consistent trends over time for multiple measures of precipitation or temperature between versions (unpublished data). We visualized the results of each PERMANOVA using non-metric multidimensional scaling (NMDS) plots with vectors for the species in each mixture and hull volumes representing the different versions (Figure S1).

To determine if species and seeding rate changes affected mixture evenness, we calculated Pielou’s evenness for each mixture for each year for both fall and spring biomass. With this metric, a more even community is one in which all species have relatively similar abundances. We ran generalized linear mixed models with Pielou’s evenness as the response variable and the interaction of study version and sampling time (fall or spring) as the predictor. All models were run using the glmmTMB package (Brooks et al., 2017) with the Tweedie distribution and Strip included as a random variable. Pielou’s evenness values were averaged by time point when constructing the stacked area plot (Figure 1).

### 3 | RESULTS

Across all mixtures, Pielou’s evenness was lower in V1 and tended to be lower in spring biomass (Figure 1). Evenness of the 4Spp and 6Spp mixtures responded to the interaction of version and sampling time ( $p = 0.045$  and  $p = 0.007$ , respectively). The evenness of the 3Spp mixture responded to both version and sampling time individually (both  $p < 0.02$ ).

#### 3.1 | Three-species mixture

Community composition of 3Spp fall biomass differed based on the interaction of version and year ( $p = 0.029$ ,  $R^2 = 0.075$ ; Table S1), but only version affected spring biomass community composition ( $p = 0.001$ ,  $R^2 = 0.308$ ). Patterns in the NMDS indicated increased CLOVER biomass drove the difference between the V1 and V2 and V3 fall biomass communities (Figure S1A,B). GRASS (rye in V1 89%, triticale in V2 62%, and V3 69%) accounted for the highest spring

biomass in all versions (Figure 1), although the amount of GRASS decreased from V1 to V2 and V3 (Figure S1B).

#### 3.2 | Four-species mixture

The community composition of both 4Spp fall and spring biomass differed based on the interaction of version and year (fall  $p = 0.048$ ,  $R^2 = 0.096$ ; spring  $p = 0.001$ ,  $R^2 = 0.194$ ; Table S1). The NMDS indicated an inverse relationship between CLOVER and the amounts of pea and GRASS, which drove the difference between the V1 and V2 and V3 communities (Figure S1C,D). In V1 and V3, GRASS had the highest biomass in the 4Spp mix (rye 72% and triticale 50%, respectively; Figure 1; Table S2), while CLOVER was the largest percentage of V2 (combined red and crimson 43%).

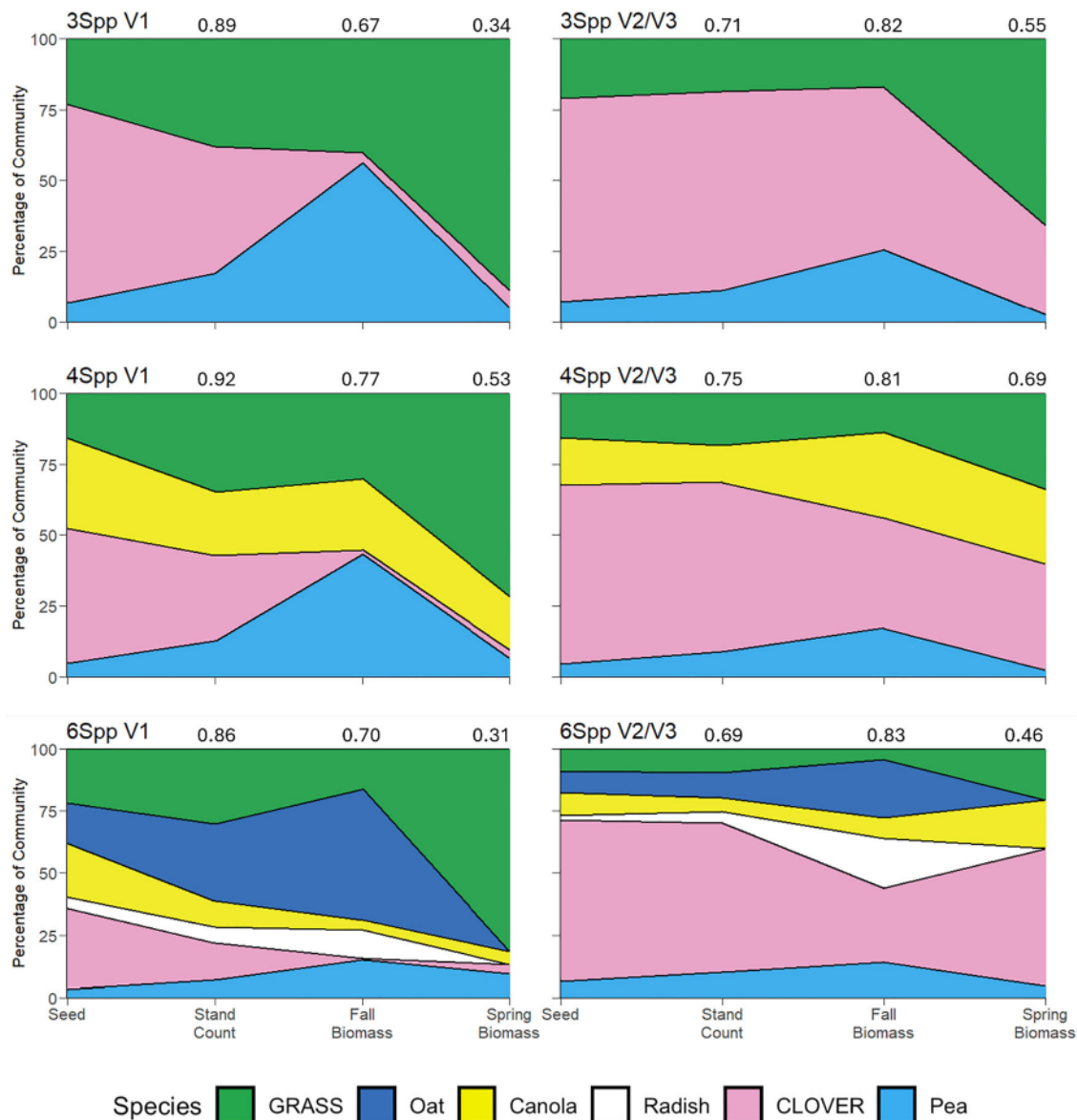
#### 3.3 | Six-species mixture

Community composition of 6Spp fall biomass differed by version ( $p = 0.001$ ,  $R^2 = 0.513$ ; Table S1), and community composition of 6Spp spring biomass differed based on the interaction of version and year ( $p = 0.002$ ,  $R^2 = 0.157$ ; Table S1). Oat was the largest percentage of V1 fall biomass (53%; Figure 1; Table S2), while CLOVER was the largest percentage of the V2 and V3 fall biomass samples (crimson clover 27% and 32%; Figure 1). An inverse relationship between the amounts of oat and GRASS and the amounts of canola and CLOVER drove the differences between the fall V1 and V2 and V3 communities (Figure S1E). V1 spring biomass samples were dominated by GRASS (rye 81%; Figure 1; Table S2), while CLOVER (crimson clover 52% and 58%) accounted for the highest spring biomass in V2 and V3 (Figure 1). The NMDS also showed that an inverse relationship between the amount of GRASS and the amount of CLOVER drove the differences between the V1 and V2 and V3 communities for 6Spp spring biomass (Figure 1F).

### 4 | DISCUSSION

Our original mixtures (V1) were not meeting key goals, due largely to poor legume performance. Here, we show that by replacing two species and adjusting seeding rates (V2 and V3), we successfully altered the community expression of three cover crop mixtures to better meet goals determined by a farmer advisory panel. version consistently had a statistically and biologically significant effect on the expression of all three cover crop mixtures.

In V1, cereal rye dominated the communities of all mixtures, making them virtually indistinguishable from one another by spring biomass sampling (Murrell et al., 2017;



**FIGURE 1** Stacked area plots depicting community composition over time. The x-axis shows four time points at which the community for each mixture was measured: initial seeding (percentage of total live seed planted), stand counts (percentage of seedling density), fall biomass, and spring biomass. Species are plotted at the mean percentage of the community they represented at each of these time points, across all years. GRASS stands for the winter-hardy grass species used (cereal rye in Version 1, triticale in Versions 2 and 3). CLOVER refers to the winter-hardy legume species used (red clover in Version 1, a mix of red and crimson clover in Version 2, and crimson clover in Version 3). Values on the top x-axis indicate the average Pielou's evenness ( $J$ ) for the community at the time of fall and spring biomass sampling. 3Spp, three-species mixture; 4Spp, four-species and five-species mixtures; 6Spp, six-species mixture; V, version.

Figure 1, left column). Cereal rye is currently the most popular cover crop species in the United States (SARE/CTIC/ASTA, 2023), in part because it can germinate later in the fall and grow later into the winter than most cover crops (Snapp et al., 2005). While these are valuable traits in a cover crop monoculture, they also enable rye to suppress other species in mixtures. Removing highly competitive cereal rye from the mixtures in our study increased the biomass of other species and the overall evenness of the mixtures (Pielou's evenness; Figure 1).

Adding crimson clover for V2 and V3 also made a difference in the expression of the cover crop communities.

Crimson clover was more productive than red clover at both fall and spring biomass sampling (Figure 1, right column), and our NMDS visualization (Figure S1) indicates that the amount of CLOVER (either high crimson biomass or low red biomass) may have been a defining characteristic for each version. Legume species are a vital source of nitrogen for organic grain producers (Blesh et al., 2019), our stakeholder audience. Replacing red clover with crimson clover shifted the distribution of species from high C:N grasses to lower C:N legumes, which likely increased nitrogen supply to the following corn crop (Finney et al., 2017). Crimson was likely

more productive than red clover because the latter is listed as a “spring seeded biennial” in our growing region, while crimson clover can be a successful winter annual here (Sustainable Agriculture Research & Education [SARE], 2010).

Although the effect of version was strong in our results, we also found several significant interactions between version and year (Figure S1). This indicates that specific characteristics of each year may also influence cover crop community expression. These results align with previous findings showing that site-specific characteristics like climate (Boniface et al., 2024) or soil nitrogen levels (Baraibar et al., 2020) can alter cover crop mixture expression.

Overall, we show selecting alternative species and adjusting seeding rates can change cover crop community composition or “expression.” The goals of our study were not unique, but where other researchers have investigated mixture composition in space via replacement series (Hayden et al., 2014; Poffenbarger et al., 2015), our long-term study is distinct in quantifying adjustments on a particular field over time. Future research with this dataset will investigate the contributions of year-specific variables (including temperature, precipitation, and soil conditions) to cover crop mixture expression to better understand the relative importance both farmer decisions and environmental conditions carry in affecting cover crop outcomes.

## AUTHOR CONTRIBUTIONS

**Sheryl C. Hosler:** Conceptualization; data curation; formal analysis; investigation; visualization; writing—original draft; writing—review and editing. **Ebony G. Murrell:** Data curation; formal analysis; visualization. **Kathleen E. Arrington:** Data curation. **Bàrbara Baraibar:** Data curation; methodology. **Mary E. Barbercheck:** Conceptualization; data curation; funding acquisition; methodology; project administration. **Brosi A. Bradley:** Data curation; investigation; methodology. **Mac Burgess:** Data curation; methodology. **Denise M. Finney:** Data curation; investigation; methodology. **Mitchell C. Hunter:** Data curation; methodology. **James C. LaChance:** Data curation. **David A. Mortensen:** Conceptualization; data curation; funding acquisition; methodology. **Charles M. White:** Conceptualization; investigation; methodology. **Carolyn J. Lowry:** Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing—review and editing. **Jason P. Kaye:** Conceptualization; funding acquisition; investigation; methodology; project administration; supervision; writing—review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

All data and code related to this manuscript will be made publicly available via ScholarSphere, Penn State’s data archive service, upon acceptance for publication.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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