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Biomass and Nitrogen Content of Hairy Vetch–Cereal Rye Cover Crop Mixtures as Influenced by Species Proportions

Hanna J. Poffenbarger, Steven B. Mirsky,* Raymond R. Weil, Jude E. Maul, Matthew Kramer, John T. Spargo, and Michel A. Cavigelli

ABSTRACT

The performance of legume–grass cover crop mixtures may be influenced by the species proportions in mixture. The objectives of this study were to: (i) evaluate total aboveground biomass and species biomass proportions resulting from different hairy vetch (legume; *Vicia villosa* Roth)/cereal rye (grass; *Secale cereale* L.) sown proportions, (ii) characterize aboveground N content and C/N ratios in response to species biomass proportions, and (iii) quantify biologically fixed nitrogen (BFN) in hairy vetch and the potential transfer of BFN to associated cereal rye. A gradient of six sown proportions ranging from 100% cereal rye to 100% hairy vetch was drilled in fall 2011 and 2012 at two sites in Beltsville, MD, and sampled for biomass, C and N content, and BFN the following spring. Hairy vetch monocultures produced less biomass than cereal rye monocultures, but biomass levels were similar between cereal rye monocultures and mixtures. Cereal rye was usually the dominant species in mixtures. Nitrogen content increased from 64 to 181 kg ha^{-1} and C/N ratio decreased from 83 to 16 as hairy vetch biomass increased from 0 to 100%. Nitrogen content was estimated to plateau when hairy vetch reached approximately 50% of the total biomass. Averaged across site-years, BFN made up 63 and 86% of hairy vetch N in monoculture and mixture, respectively. For mixtures, a wide range of sown proportions produced >8 Mg ha^{-1} biomass, but achieving maximum N content and low C/N ratios required a hairy vetch/cereal rye seeding rate of 27:34 kg ha^{-1}.

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Cover crops can provide several important services in agroecosystems, including erosion control, weed and pest suppression, nutrient retention and supply, and soil health improvement (reviewed in Snapp et al., 2005). However, individual cover crop species often vary in their capacity to achieve these functions. Small grain cover crops, such as cereal rye, grow rapidly and produce substantial biomass, making them excellent at scavenging residual inorganic N (Meisinger et al., 1991; Shipley et al., 1992), preventing erosion, and building soil organic matter (Sainju et al., 2002; Snapp et al., 2005). Cereal rye also provides reliable weed suppression as a living cover crop and after termination as a surface mulch in no-till systems (Burgos and Talbert, 1996; Akemo et al., 2000; Smith et al., 2011). However, cereal rye residues do not release substantial N during the subsequent growing season (Ranells and Wagger, 1996; Clark et al., 1997, 2007), and can even cause N immobilization during decomposition (Kuo and Sainju, 1998). Winter annual legume cover crops, such as hairy vetch, fix atmospheric N_2 and release N during decomposition (Ranells and Wagger, 1996), reducing the amount of N fertilizer required for the succeeding crop (Clark et al., 1997). However, hairy vetch grows slowly in the fall and decomposes rapidly after termination, making it less effective than cereal rye at conserving soil and suppressing weeds (Teasdale, 1993; Burgos and Talbert, 1996; Hayden et al., 2012). The rapid N release from hairy vetch residues may lead to early-season inorganic N losses before crop uptake (Rosecrance et al., 2000). Furthermore, legume cover crops such as hairy vetch can cost up to 10 times as much to establish as grasses due to their greater seed cost and weaker emergence (Snapp et al., 2005).

A hairy vetch–cereal rye cover crop mixture can merge the benefits of each component species, while attenuating their negative attributes. For example, hairy vetch–cereal rye cover crop mixtures have been shown to provide greater aboveground biomass production (Clark et al., 1994, 1997; Hayden et al., 2012), inorganic N scavenging (Tosti et al., 2014), and weed suppression (Burgos and Talbert, 1996; Hayden et al., 2012, 2014) than hairy

H.J. Poffenbarger, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011; S.B. Mirsky, J.E. Maul, and M.A. Cavigelli, USDA-ARS, Sustainable Agricultural Systems Lab., Beltsville, MD 20705; R.R. Weil, Dep. Environmental Science and Technology, Univ. of Maryland, College Park, MD 20742; M. Kramer, USDA-ARS, Statistics Group, Beltsville, MD 20705; J.T. Spargo, Agricultural Analytical Services Lab., Pennsylvania State Univ., University Park, PA 16802. *Corresponding author (steven.mirsky@ars.usda.gov).

Abbreviations: BFN, biologically fixed nitrogen; pNdfa, proportion nitrogen derived from atmosphere; RCC, relative crowding coefficient; RCCP, relative crowding coefficient product.

vetch monocultures, and greater aboveground N content than cereal rye monocultures (Clark et al., 1994; Clark et al., 2007; Parr et al., 2011; Hayden et al., 2014). The intermediate C/N ratios of hairy vetch–cereal rye mixtures contribute to more moderate N release and decomposition rates relative to hairy vetch monocultures, which can result in improved synchrony of N release and crop demand (Ranells and Wagger, 1996; Kuo and Sainju, 1998; Rosecrance et al., 2000). Some studies have even reported that hairy vetch–cereal rye mixtures can accumulate comparable or greater biomass and N relative to *both* monocultures (Clark et al., 1997; Teasdale and Abdul-Baki, 1998; Sainju et al., 2005), provide equivalent inorganic N scavenging (Tosti et al., 2014) and weed suppression (Burgos and Talbert, 1996; Teasdale and Abdul-Baki, 1998; Hayden et al., 2012) to cereal rye monocultures, and release approximately as much N to the subsequent crop as hairy vetch monocultures (Ranells and Wagger, 1996; Clark et al., 1997; Sainju et al., 2005).

Synergistic mixture effects arise because legumes and grasses can use resources in complementary ways. Legumes grown in mixture with a grass often derive a greater proportion of their N from biological N_2 fixation than in monoculture (Mallarino et al., 1990a; Izaurralde et al., 1992; Brainard et al., 2012), and the ability of legumes to biologically fix N_2 allows grasses to accumulate higher concentrations of tissue N in mixture than in monoculture (Tosti et al., 2010; Hayden et al., 2014). Biologically fixed N may even be transferred from the legume to the companion grass in mixtures either directly through mycorrhizal hyphae or indirectly through decomposition of organic tissues derived from the legume (Frey and Schüepp, 1992; Jensen, 1996). Finally, grasses and legumes differ in their aboveground architecture, allowing mixtures to capture light more efficiently than monocultures (Keating and Carberry, 1993).

A limited body of research suggests that the species proportions in mixture influence cover crop aboveground biomass, N content, N release, and effects on subsequent crop yields (Clark et al., 1994; Kuo and Sainju, 1998; Lawson et al., 2013; Hayden et al., 2014). However, few studies have explicitly measured legume–grass mixture properties across a range of sown proportions, particularly in the context of cover crop services (Clark et al., 1994; Akemo et al., 2000; Tosti et al., 2010; Brennan et al., 2011; Tosti et al., 2012; Hayden et al., 2014). Furthermore, it is known that several factors may interact with seeding rates to affect the resulting biomass proportions and mixture properties, including timing of management (Clark et al., 1994; Lawson et al., 2013), weather conditions (Ruffo and Bollero, 2003; Lawson et al., 2013), and soil N availability (Clark et al., 2007; Möller et al., 2008). To establish better management regimes for hairy vetch–cereal rye cover crop mixtures, additional research is needed to understand the effect of mixture seeding rates on cover crop properties under a range of environmental conditions.

The objectives of this study were to: (i) evaluate total aboveground biomass and species biomass proportions resulting from different hairy vetch/cereal rye sown proportions, (ii) characterize aboveground N content and C/N ratios in response to species biomass proportions, and (iii) quantify BFN in hairy vetch and the potential transfer of BFN to associated cereal rye.

MATERIALS AND METHODS

Experimental Design and Site Description

A field experiment was installed at the Beltsville Agricultural Research Center in fall 2011 and replicated in fall 2012. The experiment included six hairy vetch/cereal rye sown proportions (0:100, 20:80, 40:60, 60:40, 80:20, 100:0) planted in a randomized complete block design at each of two farms: North Farm (39.03 N, 76.93 W), which has been managed organically since 2000 and certified by the Maryland Department of Agriculture according to National Organic Program requirements since 2003, and South Farm (39.02 N, 76.94 W), which is under conventional agricultural management. The site-years in this paper are labeled "North Farm" or "South Farm" followed by the year in which the cover crops were sampled (2012 or 2013). Three replicate blocks of the experiment were planted on each site-year. Each sown proportion within each block and site-year formed a plot measuring 12 by 43 m. Soils on North Farm are classified as fine-loamy, mixed, active, mesic Fluvaquentic Endoaquepts (Hatboro series) while those on South Farm are fine-loamy, mixed, active, mesic Fluvaquentic Dystrudepts (Codorus series). The experiment succeeded the following rotation: corn (*Zea mays* L.)–sorghum-sudangrass (*Sorghum bicolor* var. *Sudanese*)–soybean [*Glycine max* (L.) Merr.]–wheat (*Triticum aestivum* L.)–soybean cover crop. The soybean cover crop was planted before experiment initiation to reduce spatial heterogeneity of soil inorganic N and to provide some residual N for the subsequent winter cover crops.

Field Management and Data Collection

The dates of field operations and sampling events are presented in Table 1. The preceding soybean cover crop was incorporated by disking in late summer each year before winter cover crop establishment. The soybeans were estimated to accumulate approximately 50 kg N ha^{-1} before termination, except on North Farm in 2013 where soybean was highly productive and accumulated approximately 90 kg N ha^{-1}. The fields were fertilized with 56 kg K ha^{-1} as K_2SO_4 and were plowed, disked, and cultimulched to prepare a cover crop seedbed. Seeding rates of each species were varied in a proportional replacement series design (Connolly, 1986), with hairy vetch/cereal rye sown proportions of: 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0. Monoculture hairy vetch and cereal rye seeding rates were 34 and 168 kg ha^{-1}, respectively, and the mixture sown proportions

Table 1. Dates of field operations and sampling events.

	2011–2012		2012–2013	
	North Farm	South Farm	North Farm	South Farm
Soybean planting	Late June–mid-July	Late June–mid-July	Late June–mid-July	Late June–mid-July
Soybean termination	Late Aug.	Late Aug.	Late Aug.	Late Aug
Cover crop planting	7 Oct.	10 Oct.	17 Sept.	25 Sept.
Soil sampling	21 Nov.	21 Nov.	12 Oct.	12 Oct.
Cover crop termination and biomass sampling	17 May	29 May	31 May	22 May

represent proportions of each species' monoculture seeding rate. Both monoculture seeding rates were selected to maximize aboveground biomass production based on preliminary research. The cereal rye monoculture seeding rate was likely higher than needed to achieve optimal biomass production, but it was selected to also provide good suppression of winter weeds (Ryan et al., 2011). The use of optimal monoculture seeding rates ensures that demands on resources remain high across all sown proportions, allowing experimental outcomes to be attributed to competitive interactions rather than to changes in total plant density (Trenbath, 1976). One thousand-seed weights for hairy vetch and cereal rye were 22.7 and 20.1 g, respectively. The prices paid for conventional hairy vetch and cereal rye seed were US\$6.61 and \$0.75 kg^{-1}, respectively. The two cover crop species were independently drilled on a 19 cm row spacing to ensure precise seeding rates across all experimental units. Hairy vetch seed was inoculated with *Rhizobium leguminosarum* biovar *viciae* before drilling covers (2.5 g inoculant kg^{-1} seed using Graph-Ex SA for cover crops, ABM, Van Wert, OH).

Soil samples to a depth of 30 cm were collected in each block after cover crop establishment each fall. Soil samples were air-dried and passed through a 2-mm sieve. Inorganic N (NO_3^-–N + NH_4^+–N) in 2-g soil subsamples was extracted with 20 mL of 1 M KCl by shaking for 1 h. Filtered extracts were analyzed colorimetrically for NO_3^-–N using Cd reduction and reaction with sulfanilamide and N-(1-Naphthyl)ethylenediamine dihydrochloride and for NH_4^+–N by reaction with salicylate and dichloro-isocyanuric acid (Seal AQ2 Automated Discrete Analyzer, Mequon, WI). Soil samples were analyzed for pH, cation exchange capacity, and Mehlich 3-extractable nutrient concentrations (P, K, Ca, Mg; Wolf and Beegle, 2011) at A&L Eastern Labs (Richmond, VA).

Cover crops were terminated using a roller/crimper in late May of 2012 and 2013. The roller/crimper consisted of a hollow steel cylinder 40 cm in diameter with blades in a chevron pattern along the outside of the cylinder designed to crush and crimp the cover crop (Ashford and Reeves, 2003). Effective termination by the roller/crimper requires that hairy vetch be flowering or developing pods (Mischler et al., 2010) and that cereal rye be at anthesis (61 on Zadoks scale) or a later growth stage (Mirsky et al., 2009). In 3 of the 4 site-years, hairy vetch was in full flower and cereal rye was in the soft dough stage (85 on Zadoks scale). On South Farm in 2013, hairy vetch was 50% flowering and cereal rye was in the milk stage (75 on Zadoks scale). Aboveground biomass was collected by clipping all material within a 76 by 67 cm frame immediately after termination in four to six locations within each plot (North Farm 2012: n = 88, South Farm 2012: n = 99, North Farm 2013: n = 61, South Farm 2013: n = 101). The biomass samples were separated by cover crop species or weeds, oven dried (60°C), weighed dry, and ground to pass a 1.0-mm screen.

Tissue C and N concentrations, and ^{15}N natural abundance were determined for each species of each sample at the Cornell University Stable Isotope Lab (Thermo Delta V Isotope Ratio Mass Spectrometer interfaced to Carlo Erba NC2500 Elemental Analyzer, Thermo Scientific, Waltham, MA; and Carlo Erba, Milan, Italy). In laboratory reporting, isotopic abundance data were expressed as $\delta^{15}N$ in parts per thousand (‰), representing the ^{15}N abundance of plant tissue relative to that of atmospheric N_2.

Soil Contamination Corrections

Cover crop sampling on South Farm occurred just after rainfall in both years, causing wet soil to adhere to the biomass samples. Therefore, hairy vetch and cereal rye dry weights and N concentrations were corrected for soil contamination following equations presented in Hunt et al. (1999) and used in a recent cover crop mixture study by Hayden et al. (2014). Subsamples of the ground plant material and six soil samples collected from the surface 5 cm of each field were heated at 550°C for 4 h to determine the ash fractions of biomass samples and of the soil that may have contaminated the biomass samples. The fraction by weight of hairy vetch or cereal rye biomass adjusted for potential soil contamination, F_{biomass}, was calculated as:

$$F_{\text{biomass}} = (A_{\text{soil}} - A_{\text{sample}})/(A_{\text{soil}} - A_{\text{plant}}) \quad [1]$$

where A_{soil} is the ash fraction of contaminating soil, A_{sample} is the ash fraction of the contaminated biomass sample, and A_{plant} is the ash fraction of uncontaminated hairy vetch or cereal rye biomass, which was estimated using clean biomass samples. Adjusted weights (M_{adj}) were calculated as:

$$M_{\text{adj}} = F_{\text{biomass}} M_{\text{sample}} \quad [2]$$

where M_{sample} is the contaminated sample dry weight. Finally, adjusted C and N concentrations and $\delta^{15}N$ values (N_{adj}) were calculated as:

$$N_{\text{adj}} = [N_{\text{sample}} - N_{\text{soil}}(1 - F_{\text{biomass}})]/F_{\text{biomass}} \quad [3]$$

where N_{sample} and N_{soil} are the C or N concentrations or $\delta^{15}N$ values of the biomass sample and soil, respectively.

Data Analyses

Cover Crop Biomass

Cover crop aboveground biomass was modeled as a function of sown proportions using plant interaction models developed by de Wit (1960):

$$y_v = y_{vv} P_v k_v/[(P_v k_v) + P_r] \quad [4]$$

$$y_r = y_{rr} P_r k_r/[(P_r k_r) + P_v] \quad [5]$$

$$y_{\text{total}} = y_v + y_r \quad [6]$$

where y_v is the hairy vetch biomass, y_{vv} is the monoculture hairy vetch biomass, P_v is the hairy vetch sown proportion, k_v is the relative crowding coefficient (RCC) of hairy vetch with respect to cereal rye, P_r is the cereal rye sown proportion, y_r is the cereal rye biomass, y_{rr} is the monoculture cereal rye biomass, and k_r is the RCC of cereal rye with respect to hairy vetch. Total aboveground biomass, y_{total}, was estimated across sown proportions as the sum of y_v and y_r. The RCC is a measure of an individual species' competitiveness: the greater the RCC value, the more productive the species was in mixture (Williams and McCarthy, 2001). An RCC value >1 indicates that the species produced more biomass when grown with the opposing species than expected based on monoculture productivity. The product of two species' relative crowding coefficient values (RCCP) provides a measure of overyielding: values >1 indicate that mixture biomass exceeded the sown proportion-weighted average of monoculture biomass, potentially due to complementary resource use (Hall, 1974). The term "overyielding" does not necessarily imply that the mixture produced greater absolute biomass than both monocultures. It is important to recognize that the interpretation of RCC and RCCP as measures of competitive ability and complementary resource use, respectively, requires that demands on resources remain high across sown proportions (Taylor and Aarssen, 1989). It should also be noted that the de Wit models do not provide accurate biomass predictions if the biomass of an individual species in mixture exceeds its monoculture biomass (Williams and McCarthy, 2001).

Fitting of the de Wit models was performed for each site-year independently using the nlme function in R (Pinheiro et al., 2013; R Development Core Team, 2014). Plot was nested within block as a random effect. We used mean monoculture biomass within each site-year to parameterize y_w and y_{rr} as constants in the model. In addition to the de Wit analysis, the effect of individual sown proportions on total aboveground biomass was determined using ANOVA (lme function; Pinheiro et al., 2013), with plot nested in block as a random effect. Means were calculated using lsmeans (Lenth, 2014) and comparisons, including a multiple comparisons adjustment ("single step") were made using glht in R package multcomp (Hothorn et al., 2008).

After checking that the de Wit model parameters were normally distributed using histograms, 95% confidence intervals were calculated for the RCC and RCCP estimates (Johnson and Kuby, 2008). To calculate confidence intervals for the RCCP estimates, we first computed the variance of each product using a first-order Taylor expansion (Goodman, 1962). The RCCs and RCCPs were declared significantly different from one when the 95% confidence interval did not overlap with one.

Nitrogen and Carbon Content

The aboveground N content of each species for each sample was computed as the product of the species' tissue N concentration and aboveground biomass. Total aboveground N content for each sample was computed as the sum of each species' N content. Although the de Wit model appeared adequate for predicting the effect of sown proportion on biomass, it did not accurately predict the effect of sown proportion on cover crop N content. Instead, linear or quadratic equations were used to model hairy vetch and cereal rye N content and total aboveground N content of cover crops as a function of hairy vetch/cereal rye biomass proportion (i.e., the proportion of hairy vetch biomass to the proportion of cereal rye biomass in each sample). Regression was performed only over the range of hairy vetch/cereal rye biomass proportions that were observed in the field to avoid extrapolating estimates for nonexistent biomass proportions. The lme function was used for regression as described for the ANOVA of total aboveground biomass, except that the explanatory variable (hairy vetch/cereal rye biomass proportion) was continuous. *Y* intercepts were fixed as either zero (for hairy vetch N content) or as the mean monoculture cereal rye N content within each site-year (for cereal rye N content and total aboveground N content).

The total C content of each sample was calculated in the same manner as total N content. The C/N ratio was computed for each sample; the ratios were natural log-transformed to meet the homogeneity of variance assumption, and then modeled as a function of hairy vetch/cereal rye biomass proportion using the same approach as used for aboveground N content.

Biological Nitrogen Fixation

The proportion of aboveground N derived from atmospheric N_2 (pNdfa) was calculated using the following equation (Shearer and Kohl, 1986):

$$\text{pNdfa} = (\delta^{15} N_{\text{ref}} - \delta^{15} N_{\text{leg}})/(\delta^{15} N_{\text{ref}} - B) \qquad [7]$$

where $\delta^{15}N_{\text{ref}}$ is the average δ^{15}N value of monoculture cereal rye samples and non-legume weeds collected at the time of termination in each block, $\delta^{15}N_{\text{leg}}$ is the δ^{15}N for hairy vetch, and *B* is the δ^{15}N of hairy vetch shoots that are fully dependent on N_2 fixation, and was approximated as −0.84‰ based on an average value for hairy vetch reported in Unkovich et al. (2008). The non-legume weed samples, dominantly shepherd's purse [*Capsella bursa-pastoris* (L.) Medik], annual bluegrass (*Poa annua* L.), and chickweed [*Stellaria media* (L.) Vill.], were collected in alleys between blocks, which had historically been managed the same as the rest of the field, but were not sown with winter cover crops. We quantified the tissue δ^{15}N of more than one reference plant for each block based on the recommendation by Unkovich et al. (2008), but the monoculture cereal rye samples had consistently lower δ^{15}N values than the non-legume weed samples. Therefore, we used an average of the two types of references (Unkovich et al., 2008) in our pNdfa calculations for both hairy vetch and cereal rye. The $\delta^{15}N_{\text{ref}}$ means for North Farm 2012, South Farm 2012, North Farm 2013, and South Farm 2013 were: 5.07, 3.57, 3.21, and 2.31‰, respectively. Cereal rye pNdfa was estimated using the same equation, substituting δ^{15}N of cereal rye for $\delta^{15}N_{\text{leg}}$ as in Moyer-Henry et al. (2006). This approach assumes that no fractionation of ^{15}N occurs during interspecific transfer.

The effect of sown proportion on hairy vetch and cereal rye pNdfa was evaluated using ANOVA as described for total aboveground biomass. One block out of three from the South Farm 2013 site-year was dropped from the analysis due to contamination of cover crop biomass by poultry litter, which was applied immediately after cover crop termination and before biomass harvest for this block only. Samples that had an ash concentration 2 to 3 percentage points greater than the mode for that species were considered highly contaminated and also excluded from the pNdfa analysis. This data processing step eliminated samples with >8% ash for cereal rye and and >12% ash for hairy vetch.

Variance Decomposition

Variance decomposition was performed for the following response variables: hairy vetch/cereal rye biomass proportion, total aboveground biomass, total aboveground N content, hairy vetch pNdfa, and cereal rye pNdfa. Categorical explanatory variables (sown proportion, field, year, block, and their two-way interactions) were all included as random effects in lmer models (R package lme4, Bates et al., 2013). The lmer outputs provided estimates of variance associated with the explanatory variables and their interactions. For each response variable, the variance percentages explained by the explanatory variables, interactions, and residuals were calculated.

RESULTS

Soil Properties and Environmental Conditions

Among the soil nutrients analyzed, K was the only nutrient that was rated as moderate rather than optimum for crop growth based on University of Maryland recommendations, and the suboptimal sufficiency levels were only observed on North Farm (Table 2). Soil inorganic N concentrations were similar in both fields within each year, and particularly high in fall 2012. Lower inorganic N concentrations in fall 2011 may have been due in part to later soil sampling in 2011 relative to 2012, which allowed time for some inorganic N to be taken up by cover crops or leached from the surface 30 cm. Soil pH ranged from 5.7 to 6.7, which is within the preferred range for hairy vetch and cereal rye.

Both 2011–2012 and 2012–2013 cover crop growing seasons were relatively mild, with growing degree day totals for most months similar to or greater than 30-yr averages (Fig. 1). The months of November and March were warmer than average in the first season, but cooler than average in the second season. Rainfall was below average for most months during both cover crop growing seasons.

The South Farm fields experienced damage by wild Canada geese in both years. Herbivory and trampling damage to cereal rye was observed in fall of 2011 on 10 to 15% of the experimental area, and goose droppings, estimated to contribute 15 to 30 kg N ha^{-1} (Scherer et al., 1995), were observed in early spring 2013.

Total Aboveground Biomass and Species Biomass Proportions

Average monoculture hairy vetch biomass ranged from 4.98 to 7.09 Mg ha^{-1} among site-years and averaged 5.92 Mg ha^{-1} across site-years (Table 3, Fig. 2). The monoculture biomass yields of cereal rye were 1.8 to 2.1 times the monoculture hairy vetch biomass yields within each site-year, ranging from 10.30 to 12.78 Mg ha^{-1} and averaging 10.95 Mg ha^{-1}. North Farm 2013 produced 1.4 times the total aboveground biomass, averaged across sown proportions, of the other 3 site-years (North Farm 2012: 9.32, South Farm 2012: 8.40, North Farm 2013: 12.38, South Farm 2013: 9.53 Mg ha^{-1}). Weed biomass in the cover crops was negligible in all site-years (site-year averages were all <12.0 kg ha^{-1}).

The de Wit model provided a good fit to the aboveground biomass of each species across sown proportions (Table 3, Fig. 2). Hairy vetch biomass generally increased as the seeding rate of hairy vetch increased, and cereal rye biomass either decreased as the seeding rate of cereal rye decreased, or remained relatively constant across the sown proportions that contained cereal rye (North Farm 2013). For North Farm 2012 and South Farm 2013, cereal rye tended to dominate the mixture biomass (cereal rye RCC estimates were 2.65 and 5.24, respectively), and hairy vetch productivity was slightly, but not significantly, lower than expected based on the sown proportions and monoculture hairy vetch productivity (hairy vetch RCC estimates were 0.98 and 0.84 for North Farm 2012 and South Farm 2013, respectively). The models predicted that equivalent biomass of cover crop species would be achieved at 80:20 to 90:10 hairy vetch/cereal rye sown proportions. The RCCP estimates for these 2 site-years were significantly >1 ($P < 0.05$; Table 3). The models did not predict that total biomass yields of mixtures would be greater than monoculture cereal rye biomass yields, but that replacing cereal rye plants with a species that produced less biomass (hairy vetch) did not result in a reduction in total aboveground biomass across mixtures. Largely consistent with the de Wit model predictions, mean total aboveground biomass at each sown proportion was close to the mean of monoculture cereal rye, except at the highest hairy vetch/cereal rye sown proportion (100:0), which produced significantly less biomass than monoculture cereal rye ($P < 0.05$; Fig. 2, top left and bottom right panels).

Relative to North Farm 2012 and South Farm 2013, cereal rye was an even greater component of mixtures on North Farm in 2013 (Fig. 3, bottom left panel). The RCC of cereal rye was significantly >1, while that of hairy vetch was significantly <1 ($P < 0.05$; Table 3). Equivalent biomass of both cover crop species was predicted around a 95:5 hairy vetch/cereal rye sown proportion. The RCCP for North Farm 2013 was marginally >1 ($P < 0.10$; Table 3). All sown proportions achieved a total aboveground biomass level similar to monoculture cereal rye, except monoculture hairy vetch, which produced significantly less biomass than monoculture cereal rye ($P < 0.05$). Although cereal rye biomass at the 40:60 hairy vetch/cereal rye sown proportion exceeded the monoculture cereal rye biomass for this site-year, the de Wit models did not accurately predict this trend.

Table 2. Soil properties of study sites for the cover crop mixture experiments at Beltsville Agricultural Research Center. Properties were measured on samples collected to 30-cm depth. Standard errors are presented in parentheses.

Soil property	2011–2012 North Farm	2011–2012 South Farm	2012–2013 North Farm	2012–2013 South Farm
USDA texture class	loam	loam	loam	loam
NO_3^-–N + NH_4^+–N, mg kg^{-1}	8.3 (0.6)	9.4 (1.1)	42.7 (3.4)	37.2 (2.1)
Total N, g kg^{-1}	0.6 (0.1)	0.4 (0.1)	0.7 (0.1)	0.7 (0.1)
Total C, g kg^{-1}	11.5 (1.1)	6.7 (0.6)	11.4 (0.9)	10.0 (1.7)
pH, 1:1 soil/water	6.7 (0.1)	6.2 (0.2)	5.7 (0.1)	5.7 (0.1)
CEC, cmol kg^{-1}	6.8 (0.4)	5.9 (0.3)	7.7 (0.1)	7.4 (0.3)
Mehlich 3-extractable				
P, mg kg^{-1}	56.7 (23.8)	67.3 (3.3)	46.0 (9.7)	76.7 (13.2)
K, mg kg^{-1}	74.7 (6.9)	93.0 (4.0)	55.0 (2.6)†	111.7 (5.2)
Ca, mg kg^{-1}	975.0 (33.1)	760.7 (60.1)	931.7 (33.4)	899.0 (8.0)
Mg, mg kg^{-1}	176.7 (5.2)	126.3 (8.9)	153.7 (2.3)	118.0 (5.5)

† Rated as moderate according to University of Maryland Soil Fertility Index Values.

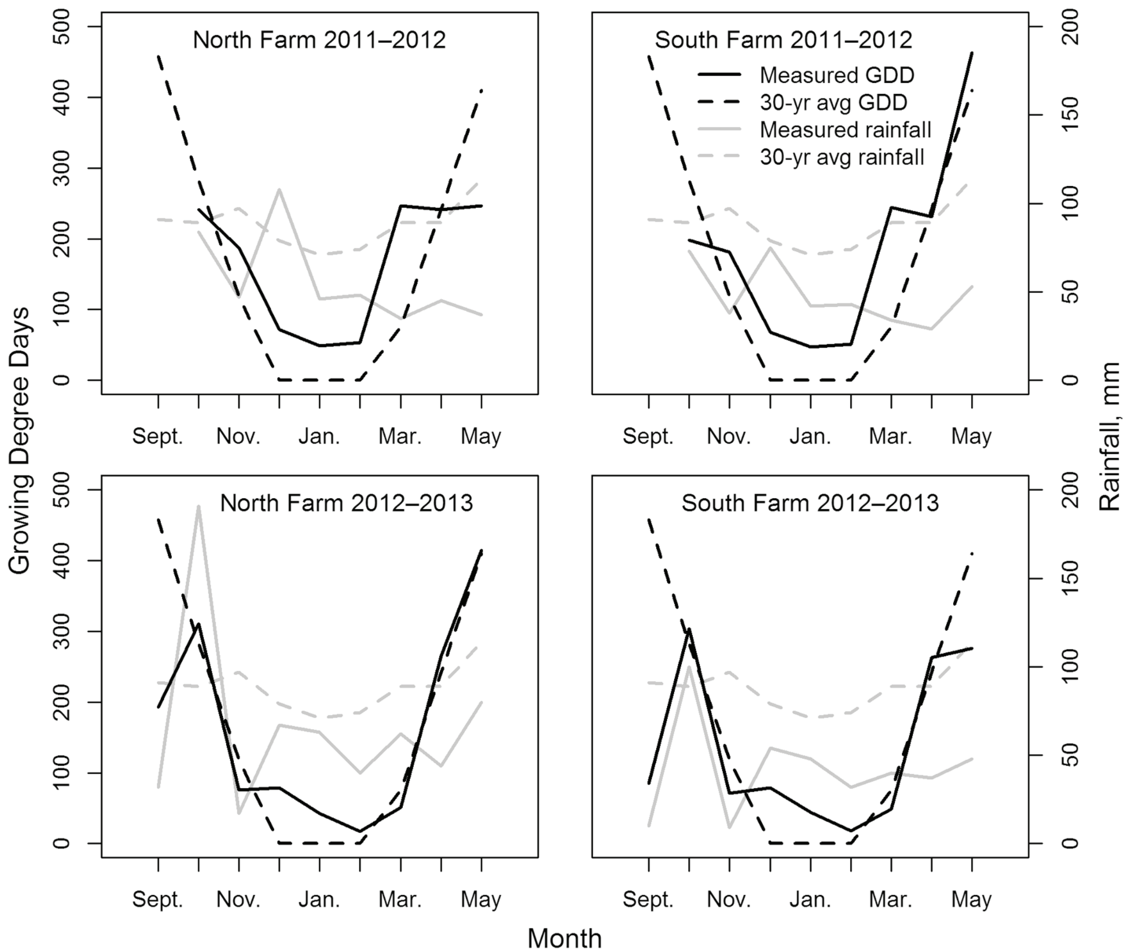

Fig. 1. Monthly growing degree days (GDD) and rainfall totals at Beltsville, MD, North Farm and South Farm for two cover crop growing seasons, 2011 to 2013. Growing degree days were computed as the average daily temperature (calculated using daily maximum and minimum temperatures) minus 4°C. Growing degree days and rainfall were summed beginning the day cover crops were planted for the month of September (2012–2013) or October (2011–2012) and ending the day cover crops were terminated for the month of May. Thirty-year averages are based on 1980 to 2010 and represent averages for the full months.

South Farm 2012 produced cover crop mixtures with a more dominant hairy vetch component, with the RCC of hairy vetch significantly >1 and that of cereal rye significantly <1 ($P < 0.05$; Table 3). Equivalent biomass of the two species was predicted around a 40:60 hairy vetch/cereal rye sown proportion. The RCCP was significantly >1 ($P < 0.05$). All sown proportions produced total aboveground biomass levels similar to monoculture cereal rye except for monoculture hairy vetch, which produced significantly less biomass than monoculture cereal rye ($P < 0.05$; Fig. 2, top right panel).

Nitrogen Content, Carbon/Nitrogen Ratios, and Biologically Fixed Nitrogen

In contrast to the biomass models, which used imposed seeding rates as the explanatory variable, N content was modeled as a function of the resulting hairy vetch/cereal rye biomass proportions in the field. The dominance of cereal rye in most of the cover crop mixtures resulted in a relatively narrow distribution of biomass proportions that could be used to predict N content in mixtures (Fig. 3). The aboveground N content of each species, total aboveground N content, and C/N ratios

Table 3. Monoculture aboveground biomass yields, relative crowding coefficients (RCCs) of hairy vetch and cereal rye, de Wit model coefficients of determination (R^2), and relative crowding coefficient products (RCCPs) for 4 site-years in Beltsville, MD. Standard errors are presented in parentheses.

	Hairy vetch			Cereal rye			
Site-year	Monoculture	RCC	R^2	Monoculture	RCC	R^2	RCCP
	Mg ha^{-1}			Mg ha^{-1}			
North Farm 2012	5.80 (0.37)	0.98 (0.21)	0.81	10.30 (0.70)	2.65 (0.54)*	0.77	2.59*
South Farm 2012	5.80 (0.29)	3.43 (0.64)*	0.81	10.31 (0.84)	0.69 (0.13)*	0.78	2.37*
North Farm 2013	7.09 (0.25)	0.22 (0.04)*	0.94	12.78 (0.84)	17.4 (6.36)*	0.79	3.91†
South Farm 2013	4.98 (0.27)	0.84 (0.15)	0.82	10.41 (0.69)	5.24 (0.79)*	0.75	4.40*

*Indicates that the RCC or RCCP value is significantly different than one ($P < 0.05$).

† Indicates that the RCCP value is marginally different than one ($P < 0.10$).

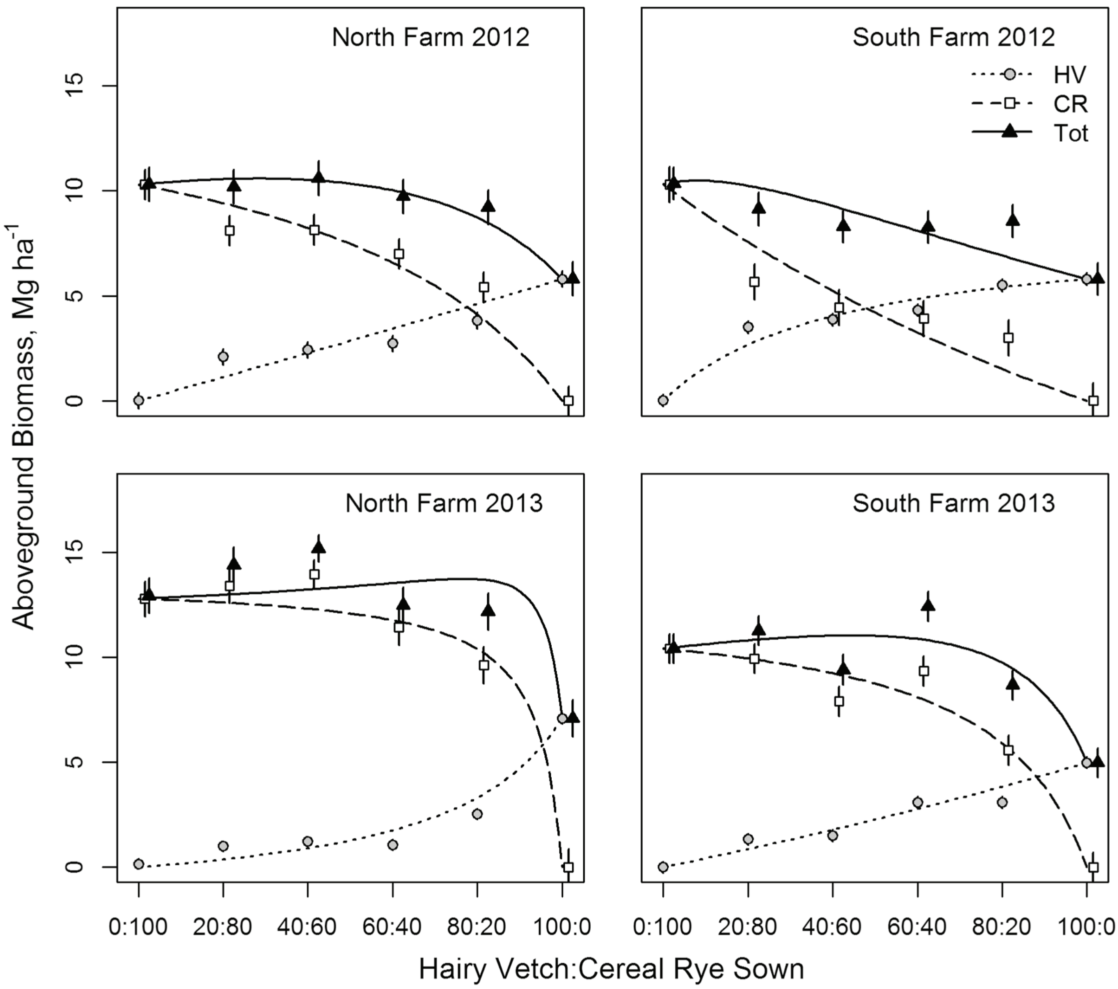

Fig. 2. Cover crop aboveground biomass in response to hairy vetch/cereal rye sown proportion for 4 site-years in Beltsville, MD. Curves represent de Wit model predictions (HV = hairy vetch, CR = cereal rye, Tot = total biomass). Error bars are ± 1 SE. Data points and error bars were offset on the *x* axis to allow better visual interpretation.

were modeled over hairy vetch/cereal rye biomass proportions of: 0:100 to 55:45 (North Farm 2012), 0:100 to 100:0 (South Farm 2012), 0:100 to 33:67 (North Farm 2013) and 0:100 to 54:46 (South Farm 2013).

Within these ranges, hairy vetch N content increased significantly with increasing proportion of hairy vetch biomass and reached maximum in monoculture hairy vetch ($P < 0.05$; Table 4, Fig. 3). Hairy vetch tissue N concentration either remained relatively constant at around 2.6% (both fields in 2012; $P > 0.05$) or decreased from >3.1 to 2.4% (both fields in 2013; $P < 0.05$) with increasing hairy vetch/cereal rye biomass proportion (data not shown). Average N content of monoculture hairy vetch ranged from 145 to 223 kg ha^{-1} among site-years, and averaged 181 kg ha^{-1} across site-years, while those of cereal rye monocultures ranged from 49 to 90 kg ha^{-1}, and averaged 64 kg ha^{-1} (Table 4, Fig. 3). Cereal rye N content decreased linearly with increasing hairy vetch biomass in mixture on South Farm 2012 ($P < 0.05$), but remained relatively constant across biomass proportions for the other site-years. Cereal rye tissue N concentrations increased with increasing hairy vetch biomass proportion from approximately 0.5 to 0.9% in 2012 and 0.7 to 0.9% in 2013 ($P < 0.05$ for all site-years except North Farm 2013; data not shown).

Total aboveground N content increased significantly with increasing hairy vetch/cereal rye biomass proportion ($P < 0.05$; Table 4, Fig. 3). The maximum total N content ranged from 176 to 223 kg ha^{-1} among the site-years and averaged 192 kg ha^{-1} across site-years, which was slightly higher than the average N content of hairy vetch monocultures. For South Farm 2012 and South Farm 2013, the maximum total N content was predicted at hairy vetch/cereal rye biomass proportions of 92:8 and 54:46, respectively. For the remaining 2 site-years, the maximum total N content was achieved in the monoculture hairy vetch treatments, but the total N content of hairy vetch–cereal rye mixtures approached the maximum total N content at the highest hairy vetch/cereal rye proportions achieved in mixture. Therefore, it appears that, if data were available over the full range of biomass proportions for all site-years, the relationships between hairy vetch/cereal rye biomass proportions and total N content would be curvilinear and would begin to plateau at around 50% hairy vetch biomass (Fig. 3).

The natural log of cover crop C/N ratios declined significantly and in a curvilinear fashion with increasing hairy vetch/cereal rye biomass proportion (Table 5, Fig. 4). Averaged across site-years, monoculture hairy vetch and cereal rye C/N ratios were 16 and 83, respectively (back-transformed from log scale). A C/N ratio of 25 to 30, which represents the soil N immobilization/mineralization threshold (Clark et al., 1997; Kuo et al., 1997), was predicted at approximately 50:50 hairy vetch/cereal

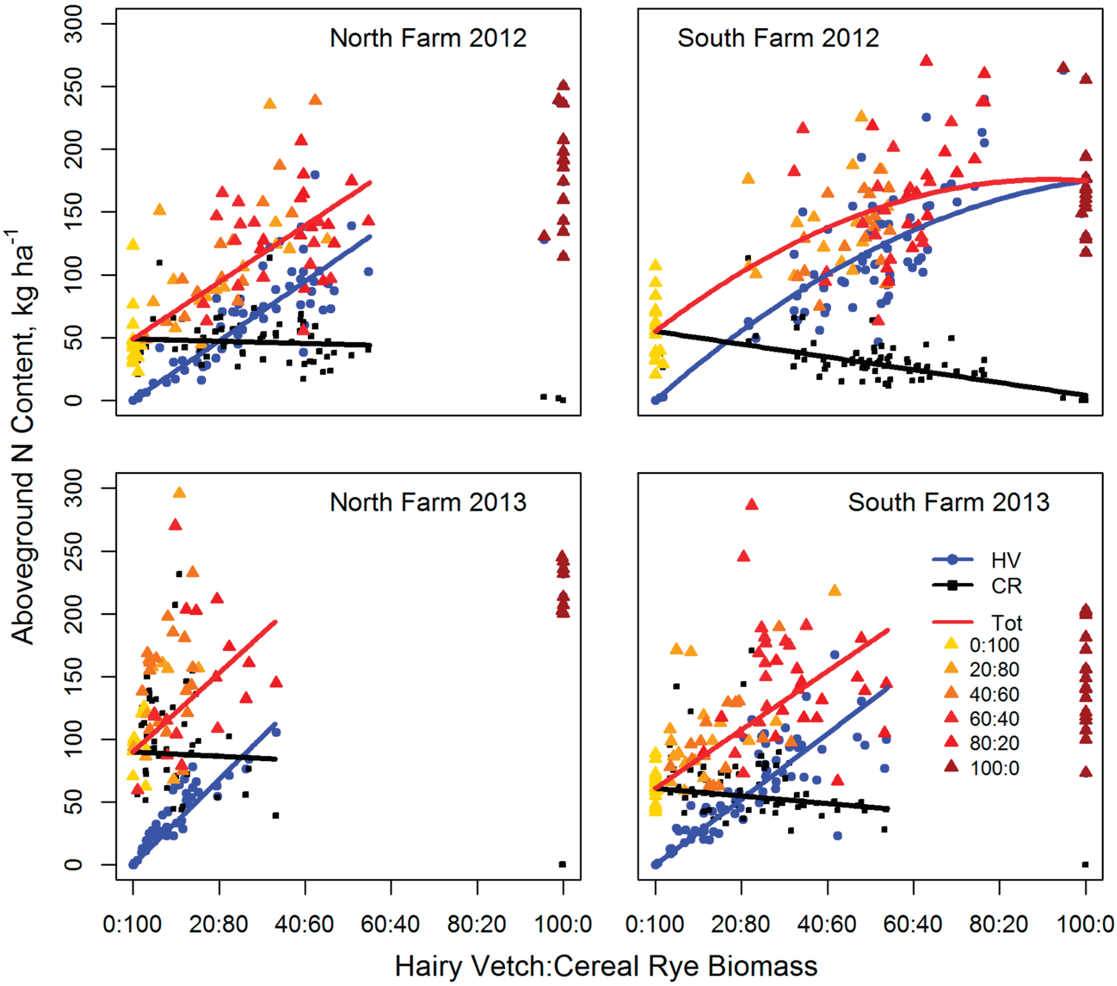

Fig. 3. Cover crop aboveground N content in response to hairy vetch/cereal rye biomass proportion for 4 site-years in Beltsville, MD. Curves represent linear or quadratic model predictions (HV = hairy vetch, CR = cereal rye, Tot = total N content). Triangles, representing total N content, were color-coded to designate the hairy vetch/cereal rye sown proportion of each sample. Models were fit only over the range of hairy vetch/cereal rye biomass proportions for which N data were available.

rye biomass proportion in the 3 site-years that achieved this biomass proportion.

Average hairy vetch pNdfa ranged from 0.70 to 0.96 in mixture and from 0.26 to 0.81 in monoculture among site-years (Table 6). There were few significant differences in hairy vetch pNdfa among mixture sown proportions within each site-year. For North Farm 2012 and South Farm 2013, hairy vetch pNdfa in monoculture was significantly less than hairy vetch pNdfa in mixture for at least one mixture sown proportion, but note that only two blocks were included in the statistical analysis for South Farm 2013. Cereal rye pNdfa, averaged across sown proportions within each site-year, ranged from 0.15 to 0.25. The ANOVA indicated that there was no effect of sown proportion on cereal rye pNdfa in all 4 site-years.

Sources of Variation

The majority (78%) of the variance associated with hairy vetch/cereal rye biomass proportion across all 4 site-years was explained by sown proportion (Table 7), while field, year and interactions of these factors with sown proportion explained most of the remaining variance. In contrast, sown proportion explained only 24 and 37% of total aboveground biomass and N content, respectively. Year, field and the field × year interaction explained much of the remaining variance associated with total aboveground biomass out of the variables included in the model, with 32% of the variance unexplained. For total aboveground N content, the interactions of sown proportion × field and field × year were important sources of variation, with 43% of variance unexplained. Hairy vetch pNdfa was influenced by the sown proportion × year interaction, year, sown proportion × field interaction, and year × block interaction, with 33% unexplained. The majority of variance (76%) associated with cereal rye pNdfa could not be explained by the variables included in the model.

DISCUSSION

Factors Affecting Cover Crop Productivity and Mixture Biomass Proportions

We found that total cover crop aboveground biomass ranged from approximately 5 to 15 Mg ha^{-1}, and averaged around 10 Mg ha^{-1} across site-years and sown proportions (Fig. 2). The biomass levels measured in the present study are generally high relative to those typically reported for hairy vetch monocultures, cereal rye monocultures, and hairy vetch–cereal rye mixtures in the mid-Atlantic United States (Clark et al., 1994, 1997; Teasdale and Abdul-Baki, 1998). Numerous studies have documented increasing cover crop biomass as termination date

Table 4. Parameter estimates and coefficients of determination (R^2) for cover crop aboveground N content in response to hairy vetch/cereal rye biomass proportion. Linear or quadratic models were fit only over the range of hairy vetch/cereal rye biomass proportions for which N data were available (North Farm 2012: 0:100 to 55:45, South Farm 2012: 0:100 to 100:0, North Farm 2013: 0:100 to 33:67, South Farm 2013: 0:100 to 54:46). All linear and quadratic coefficients shown are significantly different than zero (P < 0.05), unless indicated with italicized font. Standard errors are presented in parentheses.

Site-year	Hairy vetch N, kg ha^{-1}			
	Monoculture N	Linear coefficient	Quadratic coefficient	R^2
North Farm 2012	183 (11)	237 (11)	—	0.81
South Farm 2012	171 (10)	302 (18)	−127 (23)	0.78
North Farm 2013	223 (7)	340 (19)	—	0.89
South Farm 2013	145 (7)	261 (15)	—	0.83
	Cereal rye N, kg ha^{-1}			
	Monoculture N	Linear coefficient		R^2
North Farm 2012	49 (5)	*−9 (11)*		0.10
South Farm 2012	55 (5)	−51 (3)		0.62
North Farm 2013	90 (15)	*−17 (65)*		0.32
South Farm 2013	61 (7)	*−30 (19)*		0.40
	Total N, kg ha^{-1}			
	Linear coefficient	Quadratic coefficient		R^2
North Farm 2012	226 (20)	—		0.53
South Farm 2012	264 (23)	−144 (28)		0.54
North Farm 2013	314 (82)	—		0.38
South Farm 2013	233 (31)	—		0.63

Table 5. Parameter estimates and coefficients of determination (R^2) for natural log of cover crop C/N ratio in response to hairy vetch/cereal rye biomass proportion. Quadratic models were fit only over the range of hairy vetch/cereal rye biomass proportions for which N data were available (North Farm 2012: 0:100 to 55:45, South Farm 2012: 0:100 to 100:0, North Farm 2013: 0:100 to 33:67, South Farm 2013: 0:100 to 54:46). All linear and quadratic coefficients are significantly different than zero (P < 0.05). Standard errors are presented in parentheses.

Site-year	Natural log of C/N ratio				
	Hairy vetch monoculture	Cereal rye monoculture	Linear coefficient	Quadratic coefficient	R^2
North Farm 2012	2.72 (0.08)	4.63 (0.08)	−5.21 (0.27)	5.46 (0.70)	0.89
South Farm 2012	2.80 (0.06)	4.50 (0.06)	−3.20 (0.12)	1.52 (0.13)	0.91
North Farm 2013	2.66 (0.08)	4.15 (0.07)	−4.59 (0.77)	7.90 (2.88)	0.68
South Farm 2013	2.78 (0.05)	4.38 (0.05)	−4.32 (0.30)	4.75 (0.69)	0.90

Table 6. Proportion of N derived from the atmosphere (pNdfa) in hairy vetch and cereal rye for 4 site-years in Beltsville, MD. Significant differences in hairy vetch pNdfa among sown proportions (hairy vetch/cereal rye) within each site-year are indicated with different lowercase letters (P < 0.05; differences significant at P < 0.10 are indicated with an apostrophe). Cereal rye pNdfa was averaged over sown proportions because means were statistically equivalent among sown proportions. All means were significantly different than zero (P < 0.05), unless indicated with italicized font. Standard errors are presented in parentheses.

Site-year	Hairy vetch pNdfa					Cereal rye pNdfa
	20:80	40:60	60:40	80:20	100:0	All proportions
North Farm 2012	0.87 (0.02)ab	0.89 (0.02)a’	0.91 (0.02)a	0.86 (0.02)ab	0.80 (0.03)b	0.15 (0.04)
South Farm 2012	0.85 (0.07)a	0.91 (0.07)a	0.96 (0.07)a	0.86 (0.07)a	0.81 (0.08)a	*0.16 (0.15)*
North Farm 2013	0.88 (0.07)a	0.83 (0.06)a	0.87 (0.07)a	0.88 (0.07)a	0.64 (0.07)a	0.20 (0.05)
South Farm 2013†	0.70 (0.10)ab	0.83 (0.10)a’	0.80 (0.10)a’	0.88 (0.10)a	0.26 (0.11)b	*0.25 (0.34)*

† For South Farm 2013, one of the three blocks was not included in the statistical analysis.

Table 7. The percentage of total variance explained by experimental factors and their two-way interactions for hairy vetch/cereal rye biomass proportion, total aboveground biomass, total aboveground N content, hairy vetch proportion N derived from the atmosphere (pNdfa) and cereal rye pNdfa.

Factor	Biomass proportion	Total biomass	Total N	Hairy vetch pNdfa	Cereal rye pNdfa
	%				
Sown prop†	78	24	37	4	0
Field	3	9	0	0	0
Year	6	12	0	12	0
Block	0	2	0	0	0
Sown prop × field	3	4	8	9	0
Sown prop × year	5	6	1	20	0
Sown prop × block	0	3	1	7	1
Field × year	1	8	8	5	0
Field × block	0	0	1	0	5
Year × block	0	0	1	9	18
Residuals	4	32	43	33	76

† Sown prop = sown proportion.

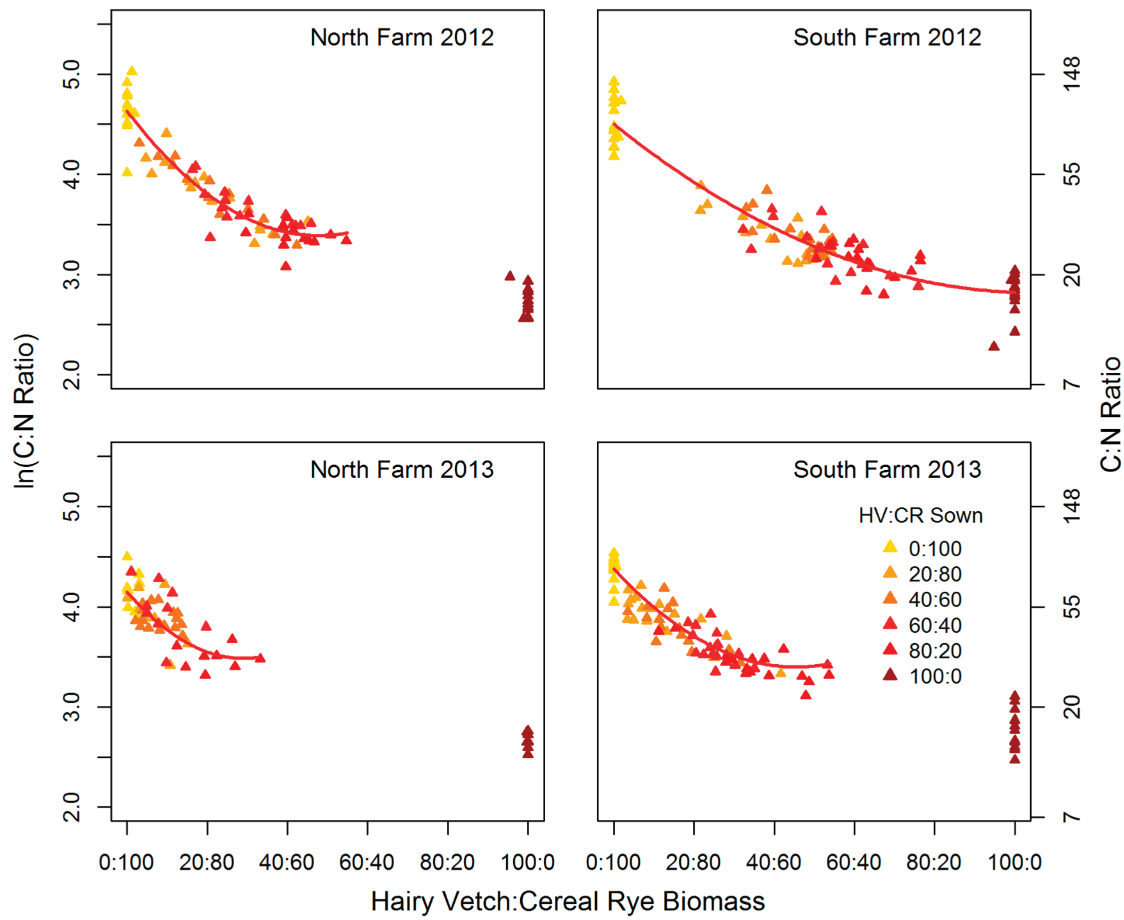

Fig. 4. Natural log of cover crop C/N ratio in response to hairy vetch/cereal rye biomass proportion for 4 site-years in Beltsville, MD. Curves represent quadratic model predictions. Triangles were color-coded to designate the hairy vetch/cereal rye sown proportion of each sample. Models were fit only over the range of hairy vetch/cereal rye biomass proportions for which N data were available.

is delayed in the spring (Clark et al., 1997; Mirsky et al., 2011), suggesting that the late termination date in the present study (as required for effective roller/crimper use) resulted in the relatively high biomass production. While the hairy vetch seeding rates used in the present study are comparable to those used in other cover crop mixture studies, our cereal rye seeding rates tended to be relatively high (Clark et al., 1994, 1997; Teasdale and Abdul-Baki, 1998). However, a high cereal rye seeding rate does not necessarily lead to greater cover crop productivity because cereal rye planted at low densities often produces more tillers (Ryan et al., 2011). We speculate that the relatively high biomass levels measured in this study were due to late termination along with the large pool of plant-available N provided by the soybean that were incorporated before cover crop establishment. Aboveground biomass levels as high as those measured in the present study have been achieved in the eastern United States by cereal rye monocultures and hairy vetch–cereal rye mixtures with late termination dates and high N availability (Clark et al., 2007; Mirsky et al., 2011; Reberg-Horton et al., 2012).

Although hairy vetch monocultures produced about half the aboveground biomass of cereal rye monocultures, mixtures produced at least 80% of the monoculture cereal rye biomass, and sometimes produced slightly greater biomass than monoculture cereal rye. Mixture biomass RCCP estimates consistently exceeded one, suggesting that mixtures produced greater biomass than expected based on the productivity of monocultures. Comparable or greater productivity of hairy vetch–cereal rye mixtures relative to both monocultures has been observed in several other studies as well (Clark et al., 1994, 1997; Ranells and Wagger, 1996; Kuo and Jellum, 2002; Sainju et al., 2005). However, the majority of hairy vetch–cereal rye mixture studies have used mixture seeding rates in which the sum of sown proportions of both species exceeded one (e.g., the hairy vetch mixture seeding rate was 0.75 times the monoculture hairy vetch seeding rate, while the cereal rye mixture seeding rate was 0.5 times the monoculture cereal rye seeding rate; sum = 1.25), making it difficult to assess whether enhanced mixture productivity was due to greater plant density or reduced competition in mixtures. Our results support findings by Hayden et al. (2014) that mixture biomass can equal or exceed biomass of both monocultures even when the sum of sown proportions is maintained at one.

In 3 out of 4 site-years, the grass (cereal rye) was the more productive component in mixtures, a finding that is consistent with the majority of grass–legume mixtures reviewed by Bedoussac et al. (2014). A more dominant cereal rye component has been observed in many hairy vetch–cereal rye mixture studies (some site-years of Ranells and Wagger, 1997; Ruffo and Bollero, 2003; Brainard et al., 2012; some site-years of Hayden et al., 2012; Lawson et al., 2013), but not in others (Ranells and Wagger, 1996; 1 site-year of Ranells and Wagger, 1997; Hayden et al., 2014). Assuming that the monoculture seeding rates used in the present study produced optimum biomass for each species (although this assumption was not verified for each site-year), we can attribute the dominance of cereal rye in mixtures to its greater competitiveness. First, it is possible that cereal rye gained a competitive advantage over hairy vetch during initial fall growth, and this advantage persisted throughout the spring. Studies that have investigated legume–grass mixture composition over time have measured a faster initial growth rate of the grass component, leading to a temporary or lasting competitive advantage over the legume (Andersen et al., 2007; Bedoussac and Justes, 2010). Greater cereal rye competitiveness could also be due to the high cereal rye seeding rate used in the present study, which has been shown to enhance cereal rye competitiveness against winter weeds (Ryan et al., 2011). Because grasses tend to outcompete legumes when ample soil N is available (Hauggaard-Nielsen and Jensen, 2001), a competitive advantage for cereal rye may also be attributed to a large amount of plant-available N released by the soybean incorporated before cover crop planting each year. Cold weather is expected to increase the competitiveness of cereal rye because it is more cold-hardy than hairy vetch (Teasdale et al., 2004). Therefore, the relatively cool early spring may also explain why cereal rye represented the larger component of cover crop mixtures in 2013. We observed that the dominance of cereal rye in mixtures did not necessarily correspond to significant suppression of hairy vetch (e.g., North Farm 2012 and South Farm 2013), likely because of the capacity of hairy vetch to fix atmospheric N_2 and to climb cereal rye plants to access light (Keating and Carberry, 1993).

Hairy vetch was the more dominant species in mixtures on South Farm in 2012. This was surprising, given the similar field conditions, geographical locations, planting dates, and termination dates among all 4 site-years. We noticed visually that cereal rye established more slowly and was generally less vigorous in the fall on this site-year relative to the others, perhaps due to cool and wet field conditions around the time of cover crop planting. Fall cereal rye growth was further impacted by herbivory and trampling by wild Canada geese in some areas of the field. Thus, cereal rye probably did not gain an early competitive advantage, and the warm spring may have given a competitive advantage to the less cold-hardy hairy vetch.

Nitrogen Content, Carbon/Nitrogen Ratios, and Biologically Fixed Nitrogen in Cover Crop Mixtures and Monocultures

Nitrogen contents of mixtures and monocultures measured in this study were similar to or greater than those reported in other studies (Ranells and Wagger, 1996; Clark et al., 1997, 2007; Sainju et al., 2005; Parr et al., 2011). We did not measure N content of hairy vetch and cereal rye roots, which are estimated to contain 10% of total hairy vetch N and 15% of total cereal rye N (Kuo et al., 1997). The responses of total N content and cover crop C/N ratios to hairy vetch/cereal rye biomass proportions were relatively consistent among site-years. However, the lack of hairy vetch/cereal rye biomass proportions above approximately 50:50 in 3 out of 4 site-years made it difficult to determine whether the relationships would be consistent among site-years across the full gradient of biomass proportions. It is important to recognize that the relationships between hairy vetch/cereal rye biomass proportion and total N content or C/N ratio would probably not hold across drastically different levels of total aboveground biomass because cover crop N content and C/N ratio usually increase with greater biomass (Clark et al., 1997). The finding that total aboveground N content plateaued at around 50% hairy vetch biomass in mixture is consistent with one out of 2 site-years in a study by Hayden et al. (2014). Several other studies have found the N content of hairy vetch–cereal rye mixtures to be equal to or greater than the N content of hairy vetch monocultures (Clark et al., 1994, 1997; Ranells and Wagger, 1996; Sainju et al., 2005).

Our pNdfa results indicate that hairy vetch fixed between 64 and 96% of its N for 3 of the 4 site-years. The method that we used to estimate pNdfa assumes that the reference plants have identical rooting patterns as hairy vetch, which is important because soil $\delta^{15}N$ can change with depth and time (Unkovich et al., 2008). In our study, natural spatial variability in soil $\delta^{15}N$ may have been exacerbated by the previous soybean cover crop, which would have released recently fixed N (with a lower $\delta^{15}N$ value) near the soil surface during decomposition. If, for example, hairy vetch acquired soil N from a shallower depth than the reference plants, it would have used more recently fixed N released by the soybean than the reference plants, leading to an overestimation of pNdfa. Due to potential inconsistencies in rooting patterns between the reference plants and hairy vetch, pNdfa estimates from this study are most reliably interpreted as relative rather than absolute measures of N_2 fixation. That said, our pNdfa figures are consistent with estimates of hairy vetch pNdfa in the literature, which range between 0.35 and 1.00, and fall mostly between 0.70 and 1.00 (Rochester and Peoples, 2005; Parr et al., 2011; Hayden et al., 2014). For South Farm 2013, hairy vetch pNdfa fell below this range in the monoculture treatment, even though soil properties and weather conditions were similar to North Farm 2013. Goose droppings were observed on this field in early March of 2013, which may have created a pulse of N that suppressed legume N_2 fixation (Möller et al., 2008), particularly in hairy vetch monocultures where cereal rye did not compete for soil N. Consistent with other legume–grass mixture studies, we found that hairy vetch pNdfa was greater in some mixtures than in hairy vetch monocultures, albeit in only a few cases (Izaurralde et al., 1992; Karpenstein-Machan and Stuelpnagel, 2000; Brainard et al., 2012).

We did not find evidence of BFN transfer in this study, as cereal rye pNdfa values were statistically equivalent in mixtures and monocultures within each site-year. However, the large variability in our cereal rye pNdfa results, perhaps due to spatial heterogeneity in the isotopic signature of soil N caused by patches of decomposing soybean residues, probably limited our power to detect statistical differences. Transfer of BFN in annual legume–grass mixtures has been observed in some studies (Frey and Schüepp, 1992; Jensen, 1996), and not in others (Izaurralde et al., 1992; Schipanski and Drinkwater, 2012). Nitrogen transfer from legumes to associated grasses has been detected more consistently in perennial mixtures, suggesting that more than one growing season may be required for this facilitative interaction to fully develop (Mallarino et al., 1990b; Schipanski and Drinkwater, 2012). Although we did not find evidence for BFN transfer, the fact that hairy vetch was able to fix a majority of its N allowed cereal rye plants to accumulate higher concentrations of tissue N in mixture than in monoculture, and scavenge similar levels of soil N in mixture as in monoculture in 3 of 4 site-years.

Averaged across site-years and sown proportions, 19% of cereal rye N was apparently biologically fixed. The positive cereal rye pNdfa values that were observed across all sown proportions, including cereal rye monocultures, can be explained as follows. Cereal rye pNdfa was calculated using reference values representing the average $\delta^{15}N$ of monoculture cereal rye and non-legume weeds. However, the $\delta^{15}N$ values of the cereal rye plants, grown both in monoculture and mixture, were consistently lower than those of the weed reference plants, making the cereal rye pNdfa values positive. Differences in the $\delta^{15}N$ values of monoculture cereal rye and weeds were probably due to differences in their rooting patterns.

Variability in Cover Crop Mixture Properties among Site-Years

The results of this study revealed large variability in cover crop mixture properties due to site-year effects, even among geographically neighboring locations. For example, we found that 17% of the variance associated with hairy vetch/cereal rye biomass proportion was explained by field, year, and the interactions of these variables with sown proportion. Similarly, Clark et al. (2007) and Brennan et al. (2011) observed considerable variability in the proportion of legume biomass in legume–cereal rye mixtures among site-years planted at the same seeding rates. Large variability in cover crop mixture composition among site-years may be attributed to differences in soil properties, weather, and previous management. On the other hand, several other studies have reported similar hairy vetch/cereal rye biomass proportions among site-years from a given sown proportion, although these studies collected fewer site-years of data than the present study (Ranells and Wagger, 1996; Teasdale and Abdul-Baki, 1998; Ruffo and Bollero, 2003; Hayden et al., 2014).

Management Implications

The choice of seeding rates for a hairy vetch–cereal rye mixture should depend on the desired functions of the cover crop (e.g., weed suppression, N scavenging, N release), site-specific conditions that influence species competitiveness, and the costs of other inputs, such as alternative sources of N. In this study, the cereal rye monocultures and hairy vetch–cereal rye mixtures consistently produced high biomass levels (>8 Mg ha^{-1}), even at the 80:20 hairy vetch/cereal rye sown proportion, suggesting that all sown proportions except monoculture hairy vetch could achieve reliable weed suppression following termination in a no-till system (Mohler and Teasdale, 1993; Teasdale and Mohler, 2000; Smith et al., 2011). If optimal biomass production is the primary goal of the cover crop, our results suggest that the most cost-effective sown proportion would be 0:100 hairy vetch/cereal rye because hairy vetch seed is considerably more expensive than cereal rye seed. Achieving maximum cover crop N content required at least 50% hairy vetch biomass component, which was usually produced at the 80:20 hairy vetch/cereal rye sown proportion. A cover crop mixture with 50% hairy vetch biomass would be expected to have a C/N ratio near 25 at the late termination date used in this study, which would likely avoid N immobilization and also allow for slower release of inorganic N than the hairy vetch monoculture (Ranells and Wagger, 1996). Mixture sown proportions between 80:20 and 20:80 hairy vetch/cereal rye provided a gradient of cover crop N content and C/N ratios, with a decreasing proportion of total aboveground N from BFN and decreasing seed cost as hairy vetch sown proportion decreased. Depending on the N requirements of the subsequent crop, the proportion of hairy vetch and cereal rye seed should be adjusted to supply adequate, but not excessive plant-available N. Importantly, our results suggest that site conditions influence the cost-effectiveness of cover crop mixtures. For example, when conditions favored cereal rye growth to the detriment of hairy vetch growth (e.g., North Farm 2013), the amount of BFN added by the hairy vetch was low relative to the high cost of hairy vetch seed.

CONCLUSIONS

Cereal rye monocultures produced approximately twice the aboveground biomass as hairy vetch monocultures. Despite this, mixture biomass levels were usually nearly equal to the biomass of cereal rye monocultures. Hairy vetch monocultures had two to four times the aboveground N content of cereal rye monocultures, yet our data suggest that total cover crop N content may plateau or peak between 50 and 100% hairy vetch biomass. Carbon/N ratios declined with increasing hairy vetch/cereal rye biomass proportion, dropping below the immobilization/mineralization threshold of 25 to 30 at around 50% hairy vetch biomass. Averaged across site-years, BFN made up 63 and 86% of hairy vetch N in monoculture and mixture, respectively. Overall the results suggest that, for mixtures, a wide range of sown proportions can provide high biomass production, but that maximum N content and a relatively low C/N ratio are most reliably achieved with a hairy vetch/cereal rye seeding rate of 27:34 kg ha^{-1} (80:20 sown proportion). Additional research is needed to further elucidate how soil properties, weather, and previous management drive species biomass proportions in cover crop mixtures.

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