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Alternative methods for terminating green manures in organic grain systems

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Abstract

Legume green manures (GM) are a vital source of nitrogen (N) for many organic grain systems. A common practice among organic growers is to undersow clover into a small grain, harvest the grain crop and terminate the clover stand in late fall by moldboard plowing in preparation for a cash crop the following spring. While fall plowing offers excellent clover kill, growers increasingly seek an alternative termination method that reduces tillage intensity and bare winter soil. This study, performed at two sites in Maine, evaluates three clover termination methods for kill efficacy, winter soil cover, spring soil conditions and N uptake and grain yield and protein of a subsequent test crop of hard red spring wheat (*Triticum aestivum* L., var. Glenn). Red clover (*Trifolium pratense* L.) was intercropped with spring barley (*Hordeum vulgare* L.) and terminated in late fall by moldboard plowing (PL), skim plowing (SK) or undercutting (UC). A control treatment received no clover and was fall plowed. An additional treatment, winterkilled field peas (WK), was evaluated at one site. SK, UC and WK increased soil cover relative to PL, though UC resulted in low clover kill efficacy in a wet spring and is in need of improved design. Grain yield was higher following red clover compared to the no-clover control at one site, but was unaffected by termination method. At one site, grain crude protein was higher following PL than the other treatments, indicating the possibility for more favorable timing of N availability associated with PL.

Introduction

In organic agriculture, where productivity is typically restricted by nitrogen (N) (Berry *et al.*, 2002), legume green manures (GMs) can be a viable N fertility option (Cherr *et al.*, 2006). Intercropping small grains with GMs, allows growers in regions with a limited growing season to establish a GM in field space that would otherwise be underutilized and, in the case of the widely used red clover, produce 10–108 kg ha^{-1} of aboveground biomass N by late fall (Gaudin *et al.*, 2013).

In northern growing regions, many farmers choose to terminate red clover in the fall to avoid delayed soil warming (Gaudin *et al.*, 2013) that could impact the spring planting date. Full incorporation by moldboard plowing is considered one of the most effective ways to terminate red clover without the use of herbicides (Hill and Sprague, 2020) yet leaves the soil with little to no surface residue through the winter and early spring, making it vulnerable to wind and water erosion (Lal *et al.*, 2007). Growers may therefore prefer a lower-disturbance method of termination (Pickoff *et al.*, 2021). Creamer and Dabney (2002) demonstrated that undercutting, a termination method that severs cover crop roots while leaving aboveground biomass intact, was successful in killing a range of legume cover crops in the spring in Ohio. As red clover is too vigorous to be terminated by roller-crimper (Clark, 2007), undercutting may offer a successful, low-disturbance alternative to plowing for northern growers. Another termination method, winterkill, may be successful in systems that utilize frost-intolerant GM species, such as field peas. This "climatic termination" method eliminates fall soil disturbance, retains maximum soil surface residue through the winter and has been successful in no-till vegetable systems (Lounsbury and Weil, 2015).

There is a need to investigate how fall termination methods affect the supply of N from GM residue to the subsequent crop. The termination method can impact the pattern of N release of GM residues by altering the residue particle size, as well as the degree to which residue is incorporated or left on the soil surface (Olesen *et al.*, 2009; Wortman *et al.*, 2012). Stute and Posner (1995) observed rapid decomposition of red clover buried in mesh bags in spring to simulate full incorporation, with 50% of total N released in the first 4 weeks. In contrast, termination methods that disturb less of the soil profile, and leave GM residues on the soil surface, lead to slower residue decomposition and a more gradual release of N (Van Den Bossche *et al.*, 2009). Yield and quality in small grain crops are greatly impacted by both the amount and timing of N supply (Mallory and Darby, 2013), and therefore could be influenced by the termination method. For example, high grain crude protein, an important quality parameter in bread wheat, is strongly linked to N availability during grain fill (Fowler, 2003) and could be positively affected by a more gradual delivery of N.

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This study evaluated GM termination methods for winter and early spring soil cover, GM kills efficacy, spring soil conditions, weed pressure and supply of N to a subsequent crop. We hypothesized that lower-disturbance, higher-residue forms of GM termination would lead to N release that is better timed to the N uptake needs of a subsequent wheat crop, while enhancing soil protection in the winter.

Methods

Study site and experimental design

The study was conducted between 2016 and 2018 at two sites at the University of Maine Rogers Farm Forage and Crop Research Facility (44°56′N, 68°42′W) in Old Town, Maine. Soils were a silt loam (Pushaw-Swanville complex, fine-silty, mixed, semiactive, nonacid, frigid Aeric Epiaquepts) at site 1 and very fine sandy loam (Nicholville, coarse-silty, isotic, frigid Aquic Haplorthod) at site 2.

The study was conducted as a 2-year crop sequence of barley/GM treatment in year 1 (the "GM phase") followed by a wheat test crop in year 2 (the "wheat phase"). It was preceded by tilled fallow and initiated at site 1 in 2016 and at site 2 in 2017 (Table 1).

The original experimental design was a randomized complete block with five replications and a full factorial split-plot arrangement of two treatment factors: termination method (plow, PL; skim plow, SK; and undercutter, UC) as the main plot factor and GM treatment (red clover, red clover/perennial ryegrass (*Lolium perenne* L.) mixture, and a no-clover control) as the split-plot factor. The red clover/perennial ryegrass treatment was dropped from the study due to poor ryegrass growth, leaving two GM treatments (clover and no-clover) and causing us to change our analytical approach, which shall be explained. As well, at site 2, one of the five replicates was abandoned due to heavy weed pressure and poor wheat performance. Sub-plots receiving UC were 1.8 m × 12.2 m, and those receiving PL or SK were 4.6 m × 12.2 m to accommodate wider equipment.

Management practices

Table 1 lists the dates of the major field operations. At the start of the GM phase, solid dairy manure was applied to all plots at a rate of 49 Mg ha^{-1} to achieve a target available N rate of 60 kg ha^{-1} (assuming 1st-year availabilities of 50 and 25% for the ammonium and organic N fractions, respectively) and incorporated the same day using a field cultivator. The following day, spring barley (cultivar Newdale) was planted in all plots using a conventional grain drill with single-disk openers and 17-cm row spacing at a target density of 300 live plants m^{-2}. In one set of subplots, red clover (cultivar Freedom) was seeded using a drop spreader at a rate of 11 kg ha^{-1}. Barley was harvested at maturity with a plot combine.

Three clover termination treatments were applied across the main plots prior to the first killing frost. The plow treatment (PL), employing a 3-bottom moldboard plow, disturbed soil to a depth of 20–30 cm, with complete inversion and burial of clover plants. The skim plow treatment (SK), also performed using a moldboard plow, disturbed the top 13–18 cm of soil, with partial inversion and burial of clover plants. The undercutter treatment (UC) used an experimental undercutter implement consisting of five overlappings, V-shaped flat sweeps, each 30 cm wide, staggered on two, weighted tool bars and mounted on a three-point hitch.

Table 1. Summary of major field operations in the green manure and wheat phases of the green manure termination trial conducted at two sites in Old Town, Maine

Operation	Dates	
	Site 1	Site 2
Green manure phase	2016	2017
Manure application	12 May	5 May
Barley[a]	12 May	6 May
Red clover seeding (Clover trt only)	12 May	6 May
Barley harvest	11 Aug.	3 Aug.
Field pea seeding (WK[a] trt only)	-	22 Aug.
Clover termination (Clover trt only)	2 Nov.	28 Oct.
Wheat test crop phase	2017	2018
Pre-plant disking	20 May 24 May 30 May	24 Apr. 7 May 11 May
P and K application	1 June	6 May
Wheat seeding	2 June	17 May
Cultivation	27 June	30 May
Wheat harvest	30 Aug.	9 Aug.

All operations were implemented equally in all treatments except where noted.
[a]At site 2, an additional termination treatment, winterkill, was included that consisted of field pea seeded after barley harvest, which died naturally over the winter.

At site 2 only, an additional main plot was included in each block to test another termination treatment, winterkill (WK). These plots were planted with barley but, after harvest, were mowed and disked. One subplot was planted to field pea (*Pisum sativum* L., target rate of 112 kg ha^{-1}), which was terminated by low winter temperatures, and the other subplot was left unplanted as a control.

In the spring following GM termination and before seeding the wheat test crop, one composite soil sample (20-cm depth) was obtained in each block to assess P and K levels (Hoskins, 1997). Plots were tilled with a heavy disk in three separate passes to prepare a seedbed. Bone meal (16% P, 80% estimated availability) and potassium sulfate (50% K, 100% estimated availability) were applied by hand to achieve a target availability of 11–26 kg ha^{-1} for P and 3–5% saturation for K and fertilizers were incorporated using a field cultivator. Spring wheat was planted at a target seeding rate of 380 live plants m^{-2} at site 1 and 500 live plants m^{-2} at site 2. At the three-leaf stage, plots were cultivated at site 1 and tine harrowed at site 2.

Measurements and analytical procedures

The day prior to barley harvest, the heights of six randomly selected barley and clover plants were recorded in each clover plot. Barley grain yield, moisture and test weight were measured on cleaned samples using a grain analysis computer (DICKEY-john Corp., Auburn, IL). The yield was adjusted to 135 g kg^{-1} grain moisture. A 100-g subsample was ground (2-mm). Total N concentration was determined by combustion of a 250-mg sub-subsample with a Leco CN2000 analyzer (Leco Corp., St. Joseph, MI), corrected to grain moisture of 120 g kg^{-1} and converted to grain protein concentration by multiplying by 5.7 (Am. Assoc. of Cereal Chem. Method 46-30).

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Green manure treatment characteristics were determined within a 1-week period prior to termination. Clover density was measured by counting the number of living clover plants in two, 30- × 33-cm permanent quadrats per plot. Clover and weed biomass was collected from four, 30- by 33-cm quadrats per plot by excavating plants rooted within the quadrat with a pitchfork. An effort was made to collect and retain as much fine root biomass as possible during sampling. Subsequent biomass sampling in the wheat phase avoided the location of this initial sampling. Intact plants were sorted into clover and weed fractions, and aboveground biomass was clipped 2 cm above the plant crowns. Roots were gently sprayed with water on a fine mesh screen to remove all soil. Aboveground and belowground plant fractions were dried at 60°, weighed, ground (2-mm) and total N and C concentration was determined as described above.

Spring soil cover was determined using the line transect method prior to clover regrowth (Laflen *et al.*, 1981), on 20 and 22 April at sites 1 and 2, respectively. Prior to tillage, the second measure of clover density was taken by counting living plants within the permanent quadrats and kill efficacy was calculated as the percent of pre-termination plants no longer alive. Living clover and weed biomass within these quadrats were clipped at 2 cm and collected.

Volumetric soil moisture was measured at 6.4 cm starting 1–3 weeks before wheat planting at site 1 and one week before planting at site 2 using a soil probe (ML3 ThetaProbe, Delta-T Devices Ltd., Cambridge, UK). Plant Root Simulator (PRS)® ion resin probes (Western Ag, Saskatoon, SK, Canada) were used to measure extractable inorganic N (Qian and Schoenau, 2005). At site 1, probes were installed in all PL, SK and UC plots that had received the clover treatment in a series of three consecutive burial periods that started on 7 June, 21 June and 14 July. At site 2, probes were installed in PL and UC plots only in two consecutive burial periods that started on 17 May and 6 June. Two (site 1) or four (site 2) probe pairs (one ion and one anion) were installed vertically at opposite ends of each plot between the third and fourth crop rows, with the center of the probe membrane located 10.2 cm below the soil surface. At the end of each burial period, probes were removed, rinsed with deionized H_2O and shipped to the vendor for analysis. Thermochron iButton® temperature loggers (Dallas Semiconductor, Maxim Integrated Products, Sunnyvale, CA), logging temperature every 30 min, were installed in the soil at 10.2 cm 1 week after wheat planting and removed after all PRS® probes were retrieved. Temperature means were calculated as mean daily temperature for a span of 5 days surrounding each sampling date.

Wheat plant density was determined at the three-leaf stage in four, 1-ft sections in each plot. Wheat and weed aboveground biomass were collected at Zadoks growth stages 30–32 (stem extension), 55–59 (head emergence) and 85 (soft dough, or "peak biomass") (Zadoks *et al.*, 1974), which corresponded to 5 July, 17 July and 13 August at site 1 and 20 June, 5 July and 1 August at site 2. All aboveground biomass was collected from two, 30- × 33-cm quadrats from opposite ends of each plot, squared over the 4th and 5th crop rows. On consecutive sampling dates, the sampling areas were placed at least 0.3 m from the preceding sample location. Plants were clipped at 1.3 cm above the soil surface, separated into wheat and weeds fractions, weighed, processed and analyzed for N concentration as described above. Wheat and weed N uptake was determined by multiplying aboveground biomass by % N, and plant N uptake was calculated as the sum of wheat and weed N uptake. Grain yield, test weight and crude protein were determined for wheat by the procedure described above for barley.

Soil inorganic N was sampled in the wheat phase prior to initial tillage (within 10 days of wheat seeding), at the start and end of each PRS probe burial period and at each wheat biomass sampling. Six soil cores (2 cm diameter and 20 cm depth) were taken within each sampling area, bulked by plot, mixed, sieved to 2 mm and a 200-g subsample was air-dried rapidly. A 3-g subsample was measured into a 50-mL polypropylene centrifuge tube with 30 mL of 2.0 M KCl and shaken for 1 h. The solution was filtered through 2-μ paper filters and the supernatant was analyzed for NH_4^+ and NO_3^- colorimetrically (ALPKEM model FS3000, O.I. Analytical, College Station, TX).

Statistical analysis

The experiment was originally designed to enable testing for a possible interaction between the termination method and GM quality, with the clover and clover/ryegrass treatments representing GMs of contrasting carbon to N ratios. The no-GM treatment was included to test for GM effects on the wheat test crop. Since the loss of the clover/ryegrass treatment prevented testing for the interaction, and the GM termination treatments were not relevant for the no-GM control, we altered our analytical approach and tested for GM treatment effects (clover vs. no clover) and termination treatment effects (PL vs. SK vs. UC) separately, each as a randomized complete block design trial with one factor, using one-way analysis of variance (ANOVA). The test GM treatment effects used data from PL only, and the test for termination treatment effects used clover subplots only. The sites were analyzed separately due to significant treatment by site interactions and differences in sampling times. ANOVA assumption of equal variance was tested using Levene's test, and residuals were tested for normality using the Shapiro–Wilk test. All means were separated using Fisher's protected LSD at $P = 0.05$. Log transformations were performed when data did not conform to the ANOVA assumptions. The statistical program JMP vs 14 was used to conduct analyses (JMP, SAS Institute, Cary, NC, USA).

Results and discussion

Weather

Weather conditions during the GM phases were typical of historical trends or did not impact GM performance (Table 2). The growing seasons for the wheat test crop (2017 and 2018) exhibited close to average temperatures but abnormal rainfall patterns. Higher than normal precipitation in May 2017 delayed wheat planting at site 1 and reduced the efficacy of the alternative clover termination methods. Conversely, at site 2, substantially lower than normal rainfall in 2018 allowed for timely wheat planting but led to poor wheat growth and high weed pressure.

Green manure treatment effects

Barley intercrop

Intercropping spring barley with a legume did not significantly affect grain yield or grain quality, except in one case, but would have reduced straw yield (Table 3). At site 2, where the lower crude protein was observed in clover vs no clover, low precipitation during stem extension and grain filling (Table 2) may have led to increased competition for water from the intercropped

Table 2. Monthly mean temperature and total rainfall from March to November of each year of the experiment in Old Town, Maine, compared with average climate data (1981–2010).

	2016	2017	2018	30-year average
Mean temperature, °C				
March	–	−3.5	−0.3	−1.4
April	–	6.9	4.4	5.3
May	12.2	11.4	13.2	11.4
June	15.9	17.0	15.9	16.4
July	20.2	19.2	21.0	19.7
August	19.9	18.1	20.7	18.6
September	15.6	16.6	15.0	13.9
October	9.1	12.0	6.1	7.8
November	3.8	2.1	−0.5	2.2
Rainfall, mm				
March	–	72	81	80
April	–	104	108	95
May	77	124	41	98
June	65	68	108	103
July	119	47	69	90
August	62	43	65	84
September	33	82	79	96
October	99	135	114	101
November	92	64	165	112

clover, restricting late-season N uptake (Unger and Vigil, 1998). On average, the clover grew to 26 and 21 cm at site 1 and site 2, respectively, well below the barley spikes, which averaged 70 and 84 cm, respectively (data not shown).

Pre-termination characteristics

Total aboveground dry matter biomass of the clover treatment measured just prior to termination was higher at site 1 than site 2 (Table 3) and contained a lower percentage of weed biomass (3% vs. 22%, data not shown). Clover density also was higher at site 1 relative to site 2 (247 vs. 201 plants m^{-2}), where lower mid-summer rainfall may have restricted clover establishment. At both sites, fall aboveground biomass and N content in the clover treatment were within the ranges reported by Gaudin *et al.* (2013) in their review of red clover intercropped with spring-seeded small grains and Koehler-Cole *et al.* (2017). We estimate that the clover treatment contained at least 131 kg N ha^{-1} at site 1 and 84 kg N ha^{-1} at site 2 in the aboveground and belowground portions combined, recognizing it is likely that not all root biomass was recovered, with an estimated C:N ratio of 14 at both sites.

The aboveground biomass and N content of plants growing in no-clover plots was higher than expected at site 1 due to weedy white clover (*Trifolium repens* L.) comprising a high proportion of the biomass (69%), but were still significantly lower than in the red clover treatment. At site 2, white clover was hand-weeded at the seedling stage and was less than 1% of no-clover plot weed biomass prior to termination. We estimate that, in total, biomass in the no-clover plots contributed 35 kg N ha^{-1} at site 1 and 30 kg N ha^{-1} at site 2. Aboveground biomass C:N of the clover treatment was significantly lower than that of the no-clover treatment at both sites, as expected.

The field pea treatment, planted at site 2 only, produced 2334 kg ha^{-1} of aboveground biomass and 95 kg ha^{-1} of aboveground biomass N (data not shown), values that were 31 and 64% higher, respectively, than clover planted at site 2.

Nitrogen dynamics

The clover treatment did not have a significant impact on soil inorganic N over the season at either of the two sites, despite the additional N incorporated (Table 4). However, at site 1, higher total plant N uptake (wheat + weeds) was observed following the clover treatment at head emergence and soft dough wheat stages, relative to the no-clover treatment, indicating a greater pool of available N later in the growing season following clover. A significant GM effect on plant N uptake was not observed at site 2.

Wheat performance

For most measures, the clover treatment had a positive or neutral effect on the wheat crop. At site 1, the clover treatment significantly elevated wheat biomass (head emergence and soft dough) and wheat N uptake (head emergence) compared with the no-clover treatment. The clover treatment also resulted in 47% higher yield and 8% higher grain crude protein than the no-clover treatment. These results were expected, as 44 kg ha^{-1} more total aboveground biomass N (Table 3) and approximately 42 kg ha^{-1} more belowground biomass N was incorporated into the soil in the clover treatment than in the no-clover treatment. As with yield, the observed effect of the clover treatment on grain crude protein and grain N yield likely stem largely from the additional N introduced into the system. However, non-N benefits associated with incorporated red clover, such as increased soil microbial activity and soil organic matter (Sarrantonio and Gallandt, 2003; Henry *et al.*, 2010), may also be at play.

At site 2, no significant differences in wheat biomass, wheat N uptake, or grain yield between clover and no-clover plots were observed, despite the fact that the clover treatment introduced an additional 35 kg N ha^{-1} as aboveground biomass and estimated 19 kg N ha^{-1} as belowground biomass relative to the no-clover treatment. The lack of a yield effect by the clover treatment suggests that yield was not limited by N at this site, but by another factor, possibly weeds. Late season weed pressure was high at this site, likely due to dry conditions that stunted wheat growth and allowed weeds a strong foothold. Weed biomass was 75% higher in the clover treatment than the no-clover treatment at the soft dough. It is possible that the additional N added by the clover treatment was preferentially taken up by well-established weed roots, causing a surge in weed biomass prior to wheat harvest.

Termination Treatment Effects

Soil cover, kill efficacy and spring soil conditions

The undercutter consistently resulted in significantly higher overwinter soil cover, as measured in early spring, than either SK or PL (Table 5). The WK treatment, tested only at site 2, provided equal soil cover to UC. The SK treatment increased soil cover relative to PL at site 1 but not site 2. Termination treatment had a substantial impact on clover kill efficacy at both sites, with PL providing the highest percent kill, UC producing the lowest and SK producing slightly lower or equal to PL.

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Table 3. Barley grain yield and quality, and green manure (GM) treatment biomass, total N, and C:N. *P*-values are from one-way ANOVA with 1 df

	Site 1				Site 2			
	Clover	No clover	*P*-value	CV, %	Clover	No clover	*P*-value	CV, %
Barley grain yield, kg ha^{-1}	2796	2950	0.384	8.7	4458	4918	0.452	18.6
Barley grain test weight, kg m^{-3}	638	648	0.415	26.4	649	644	0.614	23.1
Barley grain crude protein, g kg^{-1}	91	92	0.865	6.4	109	115	0.017*	1.4
GM aboveground biomass, kg ha^{-1}[a]	2456	1055	<0.001***	19.6	1782	802	<0.001***	33.3
GM aboveground total N, kg ha^{-1}	74	30	<0.001***	19.7	58	23	<0.001***	31.8
GM aboveground C:N	14	15	0.002**	5.2	13	16	0.006**	16.1
GM belowground biomass, kg ha^{-1}[b]	1726	457	–	–	854	231	–	–
GM belowground total N, kg ha^{-1}[b]	57	15	–	–	26	7	–	–
GM belowground C:N[b]	14	13	–	–	14	15	–	–

[a]In clover treatment, weeds comprised 3 and 22% of total aboveground biomass at site 1 and site 2, respectively. In no-clover treatment, volunteer white clover comprised 69 and <1% of total aboveground biomass at site 1 and site 2, respectively.
[b]Values for belowground biomass are based on a limited number of samples and were not statistically analyzed.
*,**,***Significant at $P < 0.05$, <0.01, <0.001.

Table 4. Green manure treatment effects on soil inorganic N (Ni), plant biomass, and N uptake during wheat phase, and wheat grain yield and quality

	Site1				Site 2			
	Clover	No clover	*P*-value	CV, %	Clover	No clover	*P*-value	CV, %
Soil Ni, kg ha^{-1}								
Pre-tillage[a]	27	23	0.264	19.3	20	18	0.161	10.3
Stem extension	22	18	0.080	15.2	30	33	0.387	14.7
Soft dough	11	11	0.937	17.6	8	10	0.132	12.0
Plant N uptake, kg N ha^{-1}								
Stem extension	62	46	0.090	14.8	52[b]	41[b]	0.289	6.8
Head emergence	89	61	0.023*	16.7	90	77	0.287	17.9
Soft dough	93	74	0.018*	9.1	121	113	0.694	23.6
Wheat N uptake, kg N ha^{-1}								
Stem extension	38	31	0.142	17.8	47[b]	36[b]	0.272	7.8
Head emergence	66	42	0.023*	20.3	72	61	0.272	16.7
Soft dough	78	58	0.069	18.4	88	94	0.761	28.0
Wheat biomass kg ha^{-1}								
Stem extension	938	798	0.184	15.9	941[b]	765[b]	0.419	4.8
Head emergence	2978	1984	0.009**	13.6	2638	2253	0.347	20.0
Soft dough	6458	4739	0.046*	6.9	5511	6108	0.683	32.2
Weed biomass, kg ha^{-1}								
Stem extension	502	522	0.743	19.2	97	89	0.620	20.5
Head emergence	1081	998	0.464	15.7	648	577	0.632	30.7
Soft dough	1127	1431	0.430	42.9	2433	1390	0.003**	8.6
Wheat grain yield, kg ha^{-1}	2791	1903	<0.001***	6.7	1982	2359	0.270	18.2
Wheat grain test weight, kg m^{-3}	806	810	0.509	1.0	750	764	0.015*	0.3
Wheat grain crude protein, g kg^{-1}	142	132	0.030*	2.6	165	162	0.567	3.5

P-values are from one-way ANOVA with 1 df.
[a]Pre-tillage sampling occurred either 1 day prior to or on the same day as the first spring tillage event (Table 1).
[b]Log transformation was performed for analysis; back-transformed mean value is presented.
*,**,***Significant at $P < 0.05$, <0.01, <0.001.

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Table 5. Termination treatment (PL, Plow; SK, Skim Plow; UC, Undercut; WK, winterkill) effects on overwinter soil cover, green manure (GM) kill efficacy, living spring biomass (aboveground), total plant and wheat N uptake, wheat and weed aboveground biomass, and wheat grain yield, test weight, and crude protein during the wheat phase

	Site 1					Site 2					
	PL	SK	UC	*P*-value	CV, %	PL	SK	UC	WK	*P*-value	CV, %
Overwinter soil cover, %	2c[a]	20b	84a	<0.001***	10.8	3b	5b	81a	81a	<0.001***	8.0
GM kill efficacy, %[b]	98a	85b	66c	<0.001***	8.4	100[c]	100[c]	38	100[c]	-	-
Living spring biomass, kg ha^{-1}	0[c]	535	1453	<0.001***	41.8	0[c]	0[c]	201	0[c]	-	-
Soil N_i, kg ha^{-1}											
Pre-tillage[d]	27	25	19	0.101	23.6	20	25	17	25	0.1968	21.8
Stem extension	22	18	18	0.176	19.9	30	37	33	27	0.7801	46.6
Soft dough	11	11	13	0.269	22.4	8	9	8	9	0.8279	19.5
Plant N uptake, kg N ha^{-1}											
Stem extension	62a	51b	48b	0.041*	16.1	52b	65a	50b	44b	0.009**	10.3
Head emergence	89a	64b	69b	0.014*	13.0	90[e]	100[e]	84[e]	80[e]	0.206	3.2
Soft dough	93	73	79	0.098	15.8	121	120	113	88	0.099	15.7
Wheat N uptake, kg N ha^{-1}											
Stem extension	38	32	27	0.237	18.5	47	50	40	43	0.593	22.8
Head emergence	66	53	40	0.082	29.9	72	81	68	71	0.552	18.2
Soft dough	78a	54b	51b	0.012*	18.6	88	65	84	75	0.404	22.1
Wheat biomass, kg ha^{-1}											
Stem extension	938	848	747	0.395	24.7	941	1006	847	880	0.806	26.7
Head emergence	2978	2819	2062	0.166	27.7	2683	2764	2725	2615	0.964	17.5
Soft dough	6458a	4968b	4472b	0.022*	17.2	5511	4292	5771	4285	0.149	19.4
Weed biomass, kg ha^{-1}											
Stem extension	502	589	475	0.373	22.7	97b	217a	230a	31b	0.002**	39.1
Head emergence	1081	1120	1329	0.378	24.2	648	637	653	369	0.593	19.4
Soft dough	1127b	1949a	2041a	0.003**	15.2	2433b	3906a	2198b	750c	0.003**	31.1
Grain yield, kg ha^{-1}	2782[e]	2582[e]	2257[e]	0.084	13.8	1886[e]	2119[e]	1528[e]	2060[e]	0.525	54.1
Grain test weight, kg m^{-3}	806	811	799	0.073	0.9	750	743	745	758	0.264	1.6
Grain crude protein, g kg^{-1}	142a	133b	131b	0.021*	3.3	165	171	161	166	0.435	5.0

P-values are from one-way ANOVA with 2 df at site 1 and 3 df at site 2.
[a]Within rows, means followed by the same letter are not significantly different according to LSD (0.05).
[b]The density of red clover plants in early spring as a percentage of the density prior to termination in the fall.
[c]Value based on visual estimate; not included in ANOVA.
[d]Pre-tillage sampling occurred either 1 day prior to or on the same day as the first spring tillage event (Table 1).
[e]Log transformation was performed for analysis; back-transformed mean value is presented.
*,**,***Significant at $P < 0.05$, <0.01, <0.001.

Living spring biomass generally reflected these treatment differences. In general, spring clover regrowth was higher at site 1 than site 2, largely due to wet spring soil conditions in 2017 (Table 2) that delayed field operations and wheat planting. The 100% kill achieved by PL, SK and WK and the resulting lack of living spring biomass at site 2 reflected drier spring soil conditions relative to the previous year. The drier spring at site 2 also increased the efficacy with which spring tillage killed surviving red clover plants in the UC plots as compared with site 1 (data not shown).

We observed that the undercutter severed clover roots several inches below the crown, leaving enough root biomass for plants to regenerate the following spring. This was particularly evident at site 1, where spring conditions were wet and cool. To achieve higher clover mortality in future studies, we advise severing the taproot closer to the crown and uprooting the crown by angling the undercutter blades slightly.

Soil moisture and temperature were affected by the termination method at site 1 but not site 2 (Table 6). At site 1, UC resulted in significantly higher soil moisture than PL at 3 weeks prior to first spring tillage (30 April), likely due to GM residue remaining on the soil surface after undercutting, as observed by Wortman *et al.* (2012) in their study comparing undercutting to disking. At site 1, UC also resulted in statistically lower soil temperature than PL at all three dates prior to tillage. It is unclear whether the increased soil moisture and decreased soil temperature in UC plots would have delayed planting relative to PL or

Table 6. Mean soil moisture and 5-day mean temperature measured in spring following termination (PL, Plow; SK, Skim Plow; UC, Undercut).

	PL	SK	UC	*P*-value	CV, %
Site 1 – 2017					
Mean soil moisture, %					
30 Apr.	30b[a]	32ab	40a	0.022*	14.4
5 May	31	31	36	0.125	12.1
17 May	29	27	36	0.058	16.6
8 Jun.	17	16	17	0.970	13.3
5-day mean soil temperature, °C					
30 Apr.	11.6a	11.5a	11.2b	0.009**	1.4
5 May	10.9a	10.7a	10.3b	0.002**	1.6
17 May	15.1a	14.5b	13.5c	<0.001***	2.1
8 Jun.[b]	17.0ab	17.2a	16.6b	0.028*	1.5
Site 2 – 2018					
Mean soil moisture, %					
23 Apr.	17	18	22	0.165	17.0
25 May	13	13	13	0.949	5.3
5-day mean soil temperature, °C					
23 Apr.[c]	9.0	–	9.0	0.929	3.0
25 May	16.9	16.8	16.6	0.680	3.0

[a]Within rows, means followed by the same letter are not significantly different according to LSD (0.05).
[b]Values reported at this date are the mean of 8 Jun. to 11 Jun. 2017.
[c]Values reported at this date are the mean of 23 Apr. to 27 Apr. 2018.
*,**,***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

SK because spring field operations were commenced at the same time for all treatments, but it is a clear possibility. At site 2, no differences in soil moisture or temperature were detected pre-tillage or post-planting, likely due to drier, more normal spring weather conditions, which allowed for timely planting of wheat.

Weed Biomass. Total mean weed biomass over time at each site reflected differences in weather conditions. At site 1, moderate weed pressure existed throughout the growing season (site average was 614 kg ha^{-1} at stem extension, 1213 kg ha^{-1} at head emergence and 1682 kg ha^{-1} at soft dough) while at site 2, dry spring conditions during wheat establishment stunted crop growth, allowing weeds to flourish later in the season (134 kg ha^{-1} at stem extension, 554 kg ha^{-1} at head emergence and 2143 kg ha^{-1} at soft dough). Weather conditions at site 2, and the resulting pattern of weed pressure, greatly affected yield and crop quality at this site, which shall be discussed below. High CVs for weed biomass reflect the characteristic patchiness of weed emergence, and may have masked possible treatment effects at both sites.

At site 1, the termination method affected weed biomass at the soft dough stage only, with UC and SK resulting in 73 and 81% higher weed biomass, respectively, relative to PL. At site 2, weed biomass in PL was again lower than UC (stem extension) and SK (stem extension and soft dough). Superior weed control following PL may be linked to the depth of disturbance, with fall moldboard plowing burying weed seeds deeper in the soil profile (Gruber and Claupein, 2009). At site 2, WK had substantially lower weed biomass than the other treatments, which may have resulted from the post-barley disking and seeding of field peas in these plots stimulating the germination of weed seeds that subsequently winterkilled, thereby depleting the weed seedbank.

Nitrogen dynamics

Termination treatment had a moderate effect on soil inorganic N and plant N uptake. Plant root simulator probes, in place during three consecutive, 12-day burial periods, detected no differences among termination treatments in extractable inorganic N at the 8–13 cm soil depth (data not shown). Soil inorganic N, measured from a full 0–20 cm soil profile, also revealed no effect at the <0.05 significance level of termination method at either site, though $P = 0.05$ at the start of the first probe burial period at site 1 (data not shown). At this point in the season, which was less than 1 week after wheat planting, soil inorganic N was appreciably lower in SK (53 kg ha^{-1}) and UC (49 kg ha^{-1}) relative to PL (73 kg ha^{-1}), potentially indicating an early-season burst in N mineralization from clover residue in PL plots. Also at site 1, PL led to significantly higher plant N uptake than SK and UC at both stem extension and head emergence. This effect may have been due to the high degree of soil-to-residue contact achieved at the time of termination in the PL treatment, which hastened mineralization the following season relative to the other termination treatments, as well as the significantly lower weed biomass in PL at the soft dough. At site 2, SK resulted in significantly higher total plant N uptake than PL at stem extension, which may be attributed to greater weed growth since wheat N uptake values were similar.

Wheat Performance. Wheat crop density was significantly impacted by the termination method at site 1 but not at site 2 (data not shown). At site 1, UC resulted in an 81% reduction in crop density relative to PL and SK (213 vs. 334 and 283 plants m^{-2}, respectively). This is likely due to plant material on or near the soil surface following spring tillage in UC plots impeding seed-soil contact at wheat seeding, or fresh clover residue having an allelopathic effect on emerging wheat seedlings (Ohno *et al.*, 2000). At site 2, where dry spring conditions prevented significant regrowth of clover in UC plots, there were no differences in wheat plant density among termination treatments.

Wheat N uptake and biomass were affected by termination treatment at site 1 but only later in the season, whereas differences in total plant N uptake were observed earlier. At soft dough (GS85), both parameters were significantly higher following PL relative to SK and UC. At site 2, termination had no effect on wheat N uptake or wheat biomass.

Wheat grain yields averaged 2.6 and 1.9 Mg ha^{-1} at site 1 and site 2, respectively. Yield at site 1 was within the range of yield obtained for the wheat variety Glenn in 4 years of variety trials at the same sites in Maine (2.3–3.9 Mg ha^{-1})(Mallory *et al.*, 2012). Despite a higher seeding rate and higher crop density at site 2, the yield was lower than at site 1, likely because of dry early season conditions and high late-season weed biomass, which may have also contributed to the high CVs observed in yield data from this site. Test weights also were higher at site 1 than site 2. High weed biomass during grain fill at site 2 may help to explain low test weight (Lyon and Hergert, 2012). Mean grain crude protein was lower at site 1, averaging 135 g kg^{-1}, likely due to the negative relationship between yield and protein, as documented by Fowler *et al.* (1990).

At site 1, the higher early-season plant N uptake and late-season wheat N uptake observed in PL did not translate into significantly higher yields relative to UC. Similarly, Sainju and Singh (2001) observed no significant differences in silage corn yield following different cover crop termination methods despite greater N mineralization of cover crop residue following moldboard plowing and chisel plowing vs a no-till treatment. Initial plant density differences also did not significantly affect final yield at site 1 in the present study, suggesting that sufficient tillering took place between seeding and harvest in UC plots to compensate for lower initial plant density. No differences were observed in site 2 yields, an expected result given that no differences in wheat stand counts, biomass, or N uptake were observed at this site.

Wheat harvested at both sites, regardless of treatment, surpassed the industry standard for crude protein for bread flour, 120 g kg^{-1} (Mallory and Darby, 2013). Grain crude protein was affected by termination treatment at site 1 but not at site 2. At site 1, SK and UC resulted in significantly lower grain crude protein relative to PL (by 7 and 8% lower for SK and UC, respectively), indicating that N dynamics were impacted by the termination method at this site despite no detectable differences in yield. Grain crude protein is strongly tied to soil N availability during crop growth, particularly during grain fill (Mallory and Darby, 2013). At site 1, plant N uptake at stem extension and head emergence was significantly higher in PL plots than UC or SK, as was wheat N uptake at the soft dough. Greater early- and mid-season N availability, and heightened late-season N uptake exhibited by the crop, appears to have been sufficient to significantly boost grain crude protein in PL relative to the other termination treatments. At site 2, the lack of termination treatment effects on grain protein echoes the lack of effects on wheat N uptake and grain yield.

Conclusions

Nitrogen added by the clover treatment had a positive effect on wheat biomass, yield and grain crude protein, illustrating that the use of GM benefits several aspects of successful crop production. Undercutting successfully enhanced winter soil cover at all sites, fulfilling a need for growers concerned about off-season soil erosion. Poor kill efficacy was the chief tradeoff associated with UC, leading to a high degree of spring clover regrowth and indicates that further research is needed to determine optimal undercutter design for severing red clover taproots. No yield penalty was observed for any of the higher residue termination methods, though grain crude protein was significantly higher following PL than UC and SK at site 1. While all three termination treatments produced wheat that met the industry standard for grain crude protein in bread wheat, this treatment effect at one site indicates that, in some cases, growers may sacrifice a price premium on their crop by using an alternative termination treatment.

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