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Impacts of summer-seeded legume cover crops and termination-tillage method on nitrogen availability to corn in rotation

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Summer-seeding legume cover crops can reduce erosion, mitigate nitrate leaching losses during the non-growing season, and provide bio-accumulated nitrogen (N) to the following crop. Very little information is available, however, on the most effective methods for terminating summer-seeded cover crops, or on the amount of N they can provide to subsequent crops. We therefore evaluated the impacts of selected legume cover crops and cover crop termination-tillage methods on corn (*Zea mays* L.) N credit and grain yield in a soybean (*Glycine max* Merr.) - winter wheat (*Triticum aestivum* L.) / cover crop - corn rotation over 3 consecutive years on a clay loam soil in southwestern Ontario. The cover crops were summer-seeded after wheat harvest, and included hairy vetch (*Vicia villosa* L. Roth), red clover (*Trifolium pratense* L.), white clover plus crimson clover mix (*incarnatum* and *Trifolium alba* L.), and a no cover crop control (CK). The cover crops were nested within termination-tillage method, including moldboard plow-down in fall, and herbicide spray-down in spring under strip-tillage or no-tillage then corn planting. Nitrogen fertilizer to corn was applied *via* side-dress at 200 kg N ha⁻¹ to the CKs, and at 100 kg N ha⁻¹ to the cover crops. Averaged over 3 years, above-ground biomass N level followed the pattern: hairy vetch > white clover plus crimson clover mix > red clover for fall plow-down termination. There were no significant differences in cover crop biomass N levels for spring termination. Corn response to cover crop was significantly affected by termination-tillage method and cover crop species, with poor corn stands after no-tillage, red clover and white plus crimson clover. Fall plow-down termination provided a range in N credit of 80–85 kg N ha⁻¹, which was significantly greater than the N credit under herbicide spray-down in spring. Under spring strip-tillage and no-tillage, hairy vetch produced significantly greater corn grain yields than red clover and white plus crimson clover mix.

KEYWORDS

cover crop, legumes, corn N credit, rotation, termination-tillage

1 Introduction

Efficient management of cover crops can increase soil organic matter, reduce soil erosion, conserve root-zone soil moisture, and enhance nitrogen (N) fertility (1–3). Legume winter-annual cover crops can also be a substantial source of N for the following crop in a crop rotation, especially in humid continental climates (4, 5). For example, Blevins et al. (6) found in Kentucky that hairy vetch (HV, *Vicia villosa* Roth) can supply about 76 kg N ha⁻¹ to corn and 125 kg N ha⁻¹ to grain sorghum, while Ott and Hargrove (7) found that both HV and crimson clover (CC, *Trifolium incarnatum* L.) increased average corn yields throughout Georgia. In addition, Pott et al. (8) showed that the fertilizer N replacement value of HV could reach 151 kg N ha⁻¹ for corn in low fertility soils in Brazil, and Ranells and Waggoner (9) showed that CC can provide up to 2/3 of the N requirement for corn in North Carolina.

However, the effectiveness of cover crops for increasing crop productivity and grain yields varies substantially with weather, length of growing season, soil type, crop species, and cover crop characteristics such as biomass production, C:N ratio and residue decomposition rate (10–12). For example, HV can release up to 80% of its accumulated biomass N within 5 weeks after termination, while CC releases about 60% in the same time period (9, 13, 14). Relative to other legume species, CC has slower N release, a narrower range of soil types where optimum performance is achieved, and a tendency to deplete soil moisture (15, 16). In a laboratory incubation study, Yang et al. (17) found that potentially mineralizable N (as % of initial N) was similar (71–75%) among shoots of CC, HV and red clover (RC, *Trifolium pratense* L.), but was substantially lower for CC roots (36.2%) than for HV and RC roots (53.0%). Some studies showed that cover crop monocultures produce greater biomass than mixtures (18–20), but others have shown that mixtures produced more biomass than their single species counterparts (21). Antosh et al. (22) found that cover crop mixtures containing hairy vetch did not accumulate as much N as hairy vetch grown alone. Hence, a legume monoculture, such as hairy vetch, may be a better strategy if the primary goal is to increase soil N.

The effectiveness of legume cover crops for producing biomass, sequestering carbon and fixing atmospheric N is impacted by the time and method of cover crop termination. Termination times range from early to late fall of the same year that the cover crop is planted, or from early spring to late spring of the following year (23, 24). Termination methods range from aggressive tillage (e.g. moldboard or chisel plowing) which incorporates most or all of the cover crop biomass into the soil, to roller-crimping or herbicide spray-down which leaves a layer of cover crop mulch on the soil surface with minimal incorporation (25–28). Generally

speaking, late-terminated cover crops accumulate more biomass and biomass N than early-terminated cover crops. Clark et al. (23) found that HV terminated in early to mid-April accumulated an average of 96 kg N ha⁻¹, while late May termination accumulated up to 149 kg N ha⁻¹. Parr et al. (11) found that HV terminated in mid-April accumulated 122 to 168 kg N ha⁻¹, while late May termination produced 155 to 217 kg N ha⁻¹. Coombs et al. (24) determined in an Ontario study that RC produced significantly greater plant available N in both soil and above-ground biomass when it was spring-terminated as opposed to fall-terminated. Cover crop termination *via* tillage versus crimping or spraying affects cover crop decomposition, with N release occurring faster and more completely from cover crops incorporated into the soil than from cover crops left lying on the soil surface (29). Cover crop surface mulches, on the other hand, can be very effective at suppressing weeds and conserving root zone soil and water (30–32), but may also reduce corn germination and early growth by delaying soil dry-down and warming (33, 34). For a sandy loam soil in southwestern Ontario, Yang et al. (35) obtained an early May above-ground biomass N accumulation of 240 kg N ha⁻¹ from HV, 199 kg N ha⁻¹ from RC, and 119 kg N ha⁻¹ from CC. In that study, when the biomass was incorporated into the soil by moldboard plowing in spring, HV and RC provided a fertilizer N replacement value (N credit) of 150 kg N ha⁻¹, and CC provided a fertilizer N replacement value of 139 kg N ha⁻¹. In a meta-analysis of 86 field trials under humid temperate climatic conditions, Bourgeois et al. (36) showed that cover crops increased average corn grain yields by 13% relative to no cover crop.

The “classic” soybean (*Glycine max* (L.) Merr) - winter wheat (*Triticum aestivum* L.) - corn (*Zea mays* L.) rotation is very popular in southwestern Ontario (37). Attempts to include cover crops in this rotation have thus far focused largely on surface broadcast of RC seed onto frozen ground (frost-seeding). Frost seeding has met with limited success, however, as there are often insufficient freeze-thaw cycles to incorporate the seed, thereby resulting in inconsistent RC stands (38, 39). An alternative approach may be to summer-seed RC or other cover crops into post-harvest wheat stubble when the soil is warm and dry.

We are not aware of any Ontario studies evaluating the performance of summer-seeded legume cover crops after winter wheat harvest.

The objectives of this study were consequently to determine for fine-textured soils in southwestern Ontario: i) the quantity of N accumulated in the above-ground biomass of selected legume cover crops when summer-seeded into post-harvest winter wheat stubble of a soybean-wheat-corn rotation; and ii) the impacts of cover crop species and termination method on corn N credit and corn grain yield.

2 Materials and methods

2.1 Field site and experimental design

The field trial was established in 2017 at the Honourable Eugene F. Whelan Experimental Farm, Agriculture and Agri-Food Canada, Woodslee, southwestern Ontario, Canada (42° 13'N, 82°44'W). Southern Ontario has a humid continental climate with four distinct seasons, and during the study years (2017–2020), mean annual air temperature and precipitation at the site were 9.8°C and 874 mm, respectively (Table S1).

The soil was of the Brookston series (Orthic Humic Gleysol, Canadian 40) with a clay loam texture in the top 15 cm (sand = 331 g kg⁻¹, silt = 374 g kg⁻¹, clay = 295 g kg⁻¹ via the pipette method of 41). Basic soil chemical characteristics included: pH of 6.3 (soil:water = 1:2); total carbon and total nitrogen contents of 18.8 g kg⁻¹ and 1.83 g kg⁻¹, respectively, via dry combustion (LECO CN-2000); available phosphorus of 15.0 mg kg⁻¹ via Olsen method (42); and available potassium of 142 mg kg⁻¹ via ICP-ammonium acetate extraction (43).

The cropping practice at the site for at least 4 years prior to the experiment was a corn – soybean - winter wheat/cover crop rotation. During that period, each phase of the rotation was fertilized according to standard soil test recommendations (37). For this study, the site was reorganized into three adjacent fields, allowing each phase of the corn-soybean-winter wheat rotation to be present each year. During the winter wheat phase, selected legume cover crops were planted into disked winter wheat stubble shortly after winter wheat harvest in mid to late summer of 2017, 2018 and 2019 (Table 1).

The field trial was a split-plot design with 4 replications having termination-tillage as the main plot and cover crop species as the sub-plot. The termination-tillage main treatments included: i) fall termination of the cover crop by moldboard plowing after first hard (killing) frost (mid to late November), then seed-bed preparation with secondary tillage and corn planting in the following spring (CT); ii) spring herbicide spray-termination of the cover crop, then no-till corn planting (NT); and iii) spring herbicide spray-termination of the cover crop followed by spring strip tillage, and then corn planting (ST). The cover crop split-plot treatments were randomly nested within the termination-tillage treatments, and included: i) hairy vetch (HV, seeding rate = 25 kg ha⁻¹); ii) red clover (RC, seeding rate = 12 kg ha⁻¹); iii) white clover plus crimson clover (WCCC) applied as a 50:50 mix (seeding rates = 12.5 kg ha⁻¹ for each); and iv) no cover crop treatment (CK). Hence, there were a total of 12 termination-tillage-cover crop combinations per block, i.e. (CT, NT, ST) × (WCCC, HV, RC, CK). The individual plots were 9.2 m wide by 30 m long for the cover crops (12 corn rows at 0.76 m spacing), and 6.1 m wide by 30 m long for the controls (8 corn rows at 0.76 m spacing). The clovers were broadcast on the surface and

planter chains lightly raked the soil so the seed was just under the surface. The HV was drill-planted to 10–15 mm depth with 180 mm row spacing.

All of the cover crops are considered winter-hardy in southwestern Ontario, and considerable re-growth usually occurs in the following spring if the cover crop was not intentionally terminated in the fall. It should also be noted that herbicide termination of white clover was often only partially effective, and the WCCC treatment was consequently changed in 2019 to crimson clover only (seeding rate = 25 kg ha⁻¹), which may have impacted the final (3rd) year of the study. Seeding rates used in this study followed Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) recommendations (http://www.omafra.gov.on.ca/english/crops/facts/cover_crops01/covercrops.htm).

2.2 Agronomic operations and sample collection

Detailed agronomic operations and sample collection are listed in Table 1. Accumulated above-ground biomass and biomass N in cover crops were determined from biomass samples collected by cutting the cover crop flush with the soil surface inside two demarcation frames (200 mm × 750 mm), then placing the cut material in paper bags, oven-drying (60 °C) for ~ 1 week, and then recording dry weights. Above-ground biomass and biomass N were determined in the fall from CT just before cover crop termination by moldboard plow-down to 180 mm depth (Nov. 29 2017, Nov. 19 2018, Nov. 21 2019), and in the following spring from NT and ST just before cover crop termination by herbicide spray-down (May 1 2018, May 14 2019, May 21 2020). Plow-down termination occurred in November after the first killing frost. Herbicide termination occurred in May using a mix of glyphosate (1.4 kg ha⁻¹ a.i.), 2,4-D (0.56 kg ha⁻¹ a.i.) and dicamba dimethylamine (0.6 kg ha⁻¹ a.i.). Spring tillage occurred a few days before corn planting, and included strip-tilling the ST plots using a Pluribus-V type strip tiller (Dawn Equipment, IL, USA), plus disking, harrowing and packing the CT plots.

Corn (cultivar PO506AM) was seeded (May 31 2018, June 24 2019, June 5 2020) in 0.76 m rows at approximately 76800 seeds ha⁻¹ using a Kinze no-till planter. Corn stands were determined 14 days after corn planting by counting emerged seedlings in two random 2-m row lengths per plot. Phosphorous and potassium fertilizers were applied at planting according to soil test recommendations (37). Nitrogen fertilizer (28-0-0 urea ammonium nitrate, UAN) was applied via side-dress (corn 6-leaf growth stage) at 200 kg N ha⁻¹ to the no cover crop control plots, and at 100 kg N ha⁻¹ to the cover crop plots. The N rate applied to the controls is within range of the rates typically recommended for corn production in southern

TABLE 1 Major agronomic activities.

Activity	2017-2018	2018-2019	2019-2020
Cover crop phase			
Winter wheat harvest	July 18, 2017	July 10, 2018	July 25, 2019
Cover crop seeding	July 31, 2017	July 16, 2018	Aug 7, 2019
Fall cover crop biomass collection	Nov 29, 2017	Nov 19, 2018	Nov 21, 2019
Plow-down of cover crops (CT)	Dec 1, 2017	Nov 28, 2018	Dec 6, 2019
Spring cover crop biomass collection (NT and ST)	May 1, 2018	May 14, 2019	May 21, 2020
Herbicide spray-down of cover crops (NT and ST) using Oracle @ 0.6kg/ha a.i. plus 2-4D Amine @ 0.56kg/ha a.i. plus Roundup @ 1.4 kg/ha a.i.	May 9, 2018	May 17, 2019	May 29, 2020
Corn phase			
Strip tilling before corn planting (ST)	May 29/30, 2018	June 12, 2019	June 1, 2020
Disk-harrow-pack before corn planting (CT)	May 30, 2018	June 11, 2019	June 3, 2020
Corn planting (CT, NT, ST)	May 31, 2018	June 24, 2019	June 5, 2020
Herbicide application to corn (Basagran Forte @ 0.84 kg/ha a.i.)	June 6, 2018	July 5, 2019	June 19, 2020
Corn side-dressed with 28% UAN	June 22, 2018	July 9, 2019	June 30, 2020
SPAD measurements at R1 corn stage	July 16, 2018	Aug 9, 2019	Aug 13, 2020
Corn harvest	Nov 28, 2018	Dec 13, 2019	Nov 19, 2020

Ontario (37). The cover crops received half the control N rate (i.e. 100 kg N ha⁻¹) because legume cover crops are known to accumulate about 200 kg N ha⁻¹ in biomass and roots (unpublished data), and we assumed 50% of biomass N would be available to the subsequent corn crop. Corn was harvested on Nov. 28 2018, Dec. 13 2019 and Nov. 19 2020, and grain yields determined at 15.5 wt. % seed moisture content.

Composited soil core samples (0 to 300-mm depth 17.5 mm diameter, n = 10) were collected from the corn treatments (about one month after corn planting and about two weeks after side-dress N application) to determine root zone NH₄⁺ and NO₃⁻ concentrations. The soil was occasionally too hard to sample, causing some of the collection dates to be missed or core depth to be limited to the top 150 mm. As a result, only data from top 150 mm are reported here. Corn chlorophyll status was measured at the R1 corn growth stage using a SPAD-502 leaf chlorophyll reader (Minolta Corporation, New Jersey, USA), and used as an estimate of N uptake status at the R1 corn stage.

2.3 Mineral soil nitrogen (NO₃⁻ And NH₄⁺), biomass, grain N and C analyses

Soil inorganic nitrogen (NH₄⁺-N and NO₃⁻-N) was determined *via* the KCl extraction method of Tel and Heseltine (44) using the Lachat QuikChem 8500 Analyzer (HACH, Dusseldorf, Germany). Oven-dried biomass samples and corn grain were ground to 2 mm and total C and N contents were determined using an automatic CN analyzer (CN-2000, Leco Corp., Saint Joseph, MI, USA).

2.4 Calculation of fertilizer N replacement value by cover crops

Apparent fertilizer N replacement value, FNRV_A (kg N ha⁻¹), of legume cover crop was calculated for each of the 9 cover crop-termination-tillage combinations, i.e. (CT, NT, ST) x (WCCC,

HV, RC), using:

$$FNRV_A = \left(\frac{CY_{trt}}{CY_{ck}} \times 200 \right) - 100 \quad (1)$$

where CY_{trt} (kg ha^{-1}) is corn grain yield for the selected cover crop-termination-tillage treatment combination, and CY_{ck} (kg ha^{-1}) is corn grain yield in the appropriate control, i.e. CT-CK, NT-CK or ST-CK. The numbers 200 and 100 are the amounts of fertilizer N (kg N ha^{-1}) applied to the CKs and cover crops, respectively. Hence, Eq. (1) calculates apparent corn N credit relative to the grain yield and N application rate of the controls; and as a result, $FNRV_A = 100 \text{ kg N ha}^{-1}$ is obtained when $CY_{trt} = CY_{ck}$, and $FNRV_A = 0 \text{ kg N/ha}$ is obtained when $CY_{trt} = 0.5 \times CY_{ck}$. This approach was taken because it provides a value that is both readily calculated and more relevant to grain corn producers than the usual approach, i.e. difference in total above-ground biomass N between treatment and control (45).

2.5 Statistical analyses

Analysis of variance (ANOVA) was carried out using the Statistical Analysis System (SAS, Cary, N.C.). Cover crop accumulated biomass N levels were analyzed for November and May. Biomass N, soil N, chlorophyll reading, grain yield and $FNRV_A$ were analyzed using a split-plot experimental design, with tillage-termination method treated as the main factor, cover crop type as the split factor, and year as a repeated measure. Fisher's protected least significant difference (LSD) test ($\alpha = 0.05$) was used to identify significant treatment main effects.

3 Results

3.1 Air temperature and precipitation during the study period

Mean monthly air temperature and precipitation over the four-year study (2017-2020) are shown in Table S1. Year 2017 and 2020 had slightly higher average annual air temperature ($10.4\text{-}10.5^\circ\text{C}$) than 2018 and 2019 ($9.6\text{-}9.8^\circ\text{C}$), and 2018 and 2019 had similar air temperatures relative to the long-term average (9.6°C). The soil was periodically subjected to below-freezing temperatures during winter, with 2018 and 2019 having a colder January (-4.6 to -4.7°C) than 2020 (-1.4°C in February). These temperature differences significantly impacted cover crop re-growth and biomass accumulation in the spring. Annual precipitation for the study period was 845 mm for 2017, 922 mm for 2018, 816 mm for 2019, and 855 mm for 2020, which were all relatively close (-8% to $+4\%$) to the long-term average (890 mm). Spring 2020 (April and May) was relatively dry (147 mm two-month total precipitation), whereas spring 2018 was

somewhat wet (199 mm). Spring 2019, on the other hand, was wet enough (205 mm) to cause a 3-week delay in corn planting. In contrast to spring, summer 2020 (June to September) was substantially wetter (387 mm two-month total precipitation) relative to summer 2018 (300 mm) and summer 2019 (290 mm). These rainfall differences likely affected annual corn yields and N uptake.

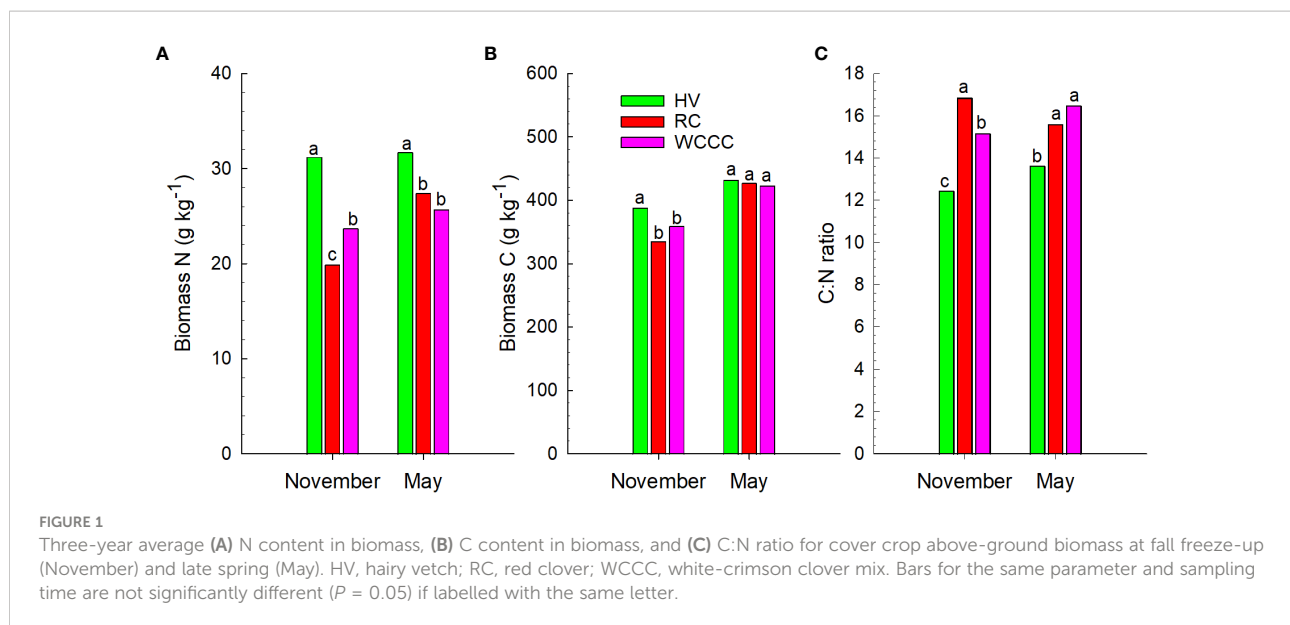
3.2 Nitrogen and carbon contents in cover crop biomass

All three cover crops established well after summer-seeding into wheat stubble, and they all achieved 100% soil coverage by fall (Figure S1). Although the selected cover crops are considered winter hardy in southwestern Ontario, partial winter kill was observed in 2018 and 2019, particularly for crimson clover and hairy vetch; and this in turn affected cover crop re-growth and biomass accumulation in the following spring. Accordingly, accumulated cover crop above-ground biomass and biomass N were reported both for the first dormancy frost in late November just before plow-down (data from CT treatment only), and also for early May just before herbicide spray-down (data from ST and NT treatments pooled).

There was substantial annual variation in above-ground biomass N and C concentrations by the cover crop treatments (Figure 1). Nitrogen concentration was higher in HV ($31.2\text{-}31.7 \text{ g N kg}^{-1}$) than in RC and WCCC ($19.9\text{-}27.4 \text{ g N kg}^{-1}$) in both fall and spring samples, and greater N concentration was found in WCCC (24.7 g N kg^{-1}) than in RC (19.9 g N kg^{-1}) in fall samples but not in spring samples (Figure 1A). All cover crops had significantly greater above-ground biomass C concentrations in spring ($423\text{-}432 \text{ g kg}^{-1}$) than in fall ($335\text{-}388 \text{ g kg}^{-1}$), and C concentration was higher in HV than in RC and WCCC in fall samples (Figure 1B). The November and May C:N ratios were 15.1 to 16.8 for RC and WCCC; however, the C:N ratio for HV was lower in November (12.4) and May (13.6) (Figure 1C).

Annual average above-ground biomass and biomass N varied among year, season and cover crop (Table 2). In 2017-2018 and 2019-2020, cover crop biomass was significantly lower for RC (4.0 Mg ha^{-1}) than HV (5.8 and 6.2 Mg ha^{-1}) and WCCC (6.2 and 7.2 Mg ha^{-1}) in November at the start of over-winter dormancy. November 2017 cover crop biomass N was RC (93 kg N ha^{-1}) < WCCC (163 kg N ha^{-1}) < HV (213 kg N ha^{-1}), and November 2018 cover crop biomass N was RC (87 kg N ha^{-1}) < WCCC (136 kg N ha^{-1}) < HV (184 kg N ha^{-1}). In 2019, November cover crop biomass N followed a different pattern with RC (85 kg N ha^{-1}) < WCCC (175 kg N ha^{-1}) = HV (182 kg N ha^{-1}).

Spring re-growth had a large impact on accumulated cover crop biomass and biomass N in the following May before herbicide spray-down. In May 2018, accumulated biomass and



biomass N was HV > RC > WCCC; in May 2020, biomass N was HV > RC = WCCC, while accumulated biomass was HV > RC only; and in May 2019 HV had lower accumulated biomass and biomass N than both RC and WCCC. Over three years, HV had the greatest and RC had the lowest accumulated biomass and biomass N at freeze-up in late November; however, these differences always disappeared by early May of the following year. It was also found that WCCC and HV exhibited appreciable over-winter die-off in 2018 (HV and WCCC) and 2019 (HV), which was likely caused by cold air temperatures during December 2017 to January 2018, and during January to February 2019 (Table S1).

3.3 Impacts of cover crop species and termination-tillage method on corn stand

Corn planting date varied with spring weather, and a substantial planting delay occurred in 2019 due to wet soil conditions (Table 1). Corn emergence in CT was fast and even for all cover crops. In NT and ST, corn emerged later, and it emerged slower for RC and WCCC than for HV, especially under NT where above-ground cover crop biomass remained on the soil surface after termination by spring herbicide spray-down (Figure S2). Corn stands at 14 days after planting varied among year, season and cover crop (Figure 2). Averaged over cover crop and year, CT produced more uniform and greater corn stands (73,960 plants ha⁻¹) than ST (69,030 plants ha⁻¹) and NT (56,870 plants ha⁻¹). In NT, 3-yr average corn stands followed the pattern: CK (72,450 plants ha⁻¹) > HV (60,700 plants ha⁻¹) > WCCC (52,220 plants ha⁻¹) > RC (42,100 plants ha⁻¹); and in ST, 3-yr average corn stands followed the trend:

CK (73,270 plants ha⁻¹) > WCCC (68,900 plants ha⁻¹) = RC (68,350 plants ha⁻¹) = HV (65,620 plants ha⁻¹). Corn stands were not significantly different, however, among the CT treatments (72,730 - 75,190 plants ha⁻¹). Compared to CT, overall average corn stand was 23% lower in NT and 6% lower in ST. In 2020, RC-NT had the lowest average corn stands (18860 plants ha⁻¹), which was 24% of the average corn stands in the corresponding no cover crop control (CK-NT). This indicates that cover crop species and termination-tillage method significantly impact both corn emergence and seedling establishment; i.e. no difference between cover crops and control for CT, but significant reduction in corn emergence and plant stands between cover crops and control for NT and ST.

3.4 Effects of cover crop species and termination-tillage method on available soil N and corn N status

There were no cover crop or tillage-termination method interaction effects for plant available soil N, so only main effects are reported (Table 3). The no cover crop controls (CK-CT, CK-NT, CK-ST) contained significantly more soil NH₄⁺-N and total soil mineral N (NO₃⁻ + NH₄⁺) than the cover crop treatments in all three years; and in 2020, HV contained significantly more soil NO₃⁻-N than RC. Significantly more soil NO₃⁻-N was also found under NT and ST (60.0 and 64.7 mg N ha⁻¹, respectively) relative to CT (44.8 mg N ha⁻¹) in 2020 (data not reported), which likely reflects greater biomass N in 2020 than in the previous two years (Table 2). Although there was more mineral N under the controls due to the higher N application rate (200 kg N ha⁻¹) than under the cover crops (100 kg N ha⁻¹), N release and

TABLE 2 Above-ground crop biomass and biomass nitrogen (N) in cover crop (HV, RC, WCCC) in November 2017, 2018 and 2019 shortly before cover crop termination by moldboard plow-down (CT treatment), and in May 2018, 2019 and 2020 shortly before cover crop termination by herbicide spray-down (NT and ST treatments).

Time	Cover crop	2017-2018		2018-2019		2019-2020	
		Biomass	N content	Biomass	N content	Biomass	N content
		Mg ha ⁻¹	kg N ha ⁻¹	Mg ha ⁻¹	kg N ha ⁻¹	Mg ha ⁻¹	kg N ha ⁻¹
November							
	HV	5.8	213	7.4	184	6.2	182
	RC	4.0	93	6.9	87	4.0	85
	WCCC	6.2	163	7.8	136	7.2	175
	LSD(0.05)	0.7	19	ns	26	1.5	20
May							
	HV	5.0	163	3.2	77	9.3	356
	RC	3.5	104	6.2	166	7.7	206
	WCCC	2.3	61	6.2	154	8.1	210
	LSD(0.05)	0.7	23	0.7	35	1.5	52

HV, hairy vetch; RC, red clover; WCCC; white-crimson clover mix. LSD (0.05) = Protected Fisher's Least Significant Difference in a *P* value of 0.05.

availability occurs throughout the growing season in the cover crop treatments, as evidenced by the plant chlorophyll SPAD readings described below.

There were also no tillage-termination method or cover crop interaction effects for corn plant chlorophyll levels (R1 growth stage) (Table 4), while the main effects of tillage-termination

method were significant in 2018 and 2020, and cover crop significantly affected chlorophyll levels in 2019 and 2020. The SPAD readings were greater for CT (55.5) than ST (53.6) in 2018, and also greater for CK (51.8) than WCCC (47.9) in 2019. In 2020, SPAD readings were greater for CT (57.4) than ST (55.3), and they were also greater for CK (58.8) than for the

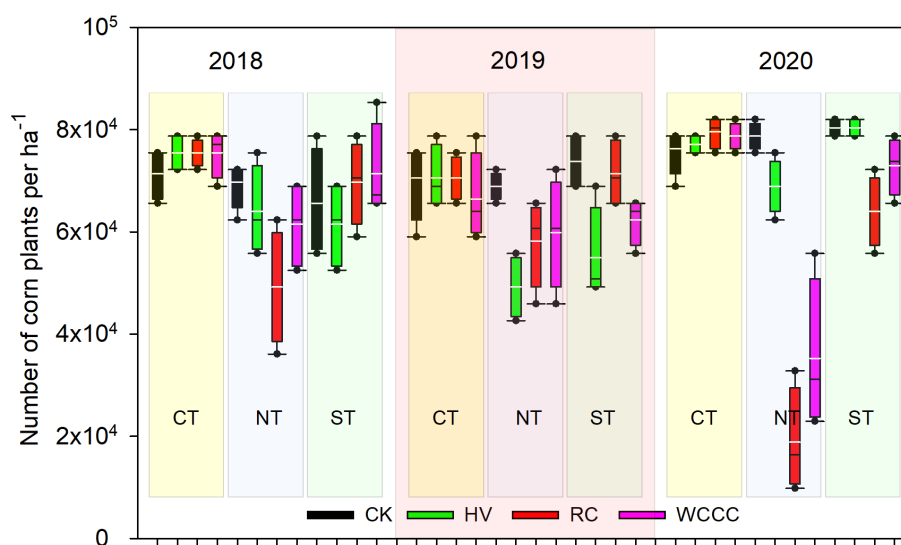


FIGURE 2 Corn stands (plant ha⁻¹, two weeks after planting) by year from 2018 to 2020 grouped by cover crop species and cover crop termination-tillage method. CT, fall moldboard plow-down termination followed by spring planting; NT, spring herbicide spray-down termination followed by no-till corn planting; ST, spring herbicide spray-down termination followed by strip tilling and corn planting; CK, no cover crop control; HV, hairy vetch; RC, red clover; WCCC, white and crimson clover mix. Boxes indicate the 25th and 75th percentile; solid dots above and below the box indicate the 90th and 10th percentile, respectively; white horizontal lines indicate mean; black horizontal lines indicate median.

TABLE 3 Effects of cover crop species and termination-tillage method on soil mineral nitrogen concentrations in the 0-150 mm depth range at one month after corn planting each year.

	2018			2019			2020		
	NO ₃ ⁻	NH ₄ ⁺	Min N	NO ₃ ⁻	NH ₄ ⁺	Min N	NO ₃ ⁻	NH ₄ ⁺	Min N
	-----mg N kg ⁻¹ -----								
CK	48.9	42.1	91.0	54.9	34.7	89.5	56.2	116	172
HV	44.3	10.9	55.2	38.6	3.5	42.1	69.3	29.0	98.3
RC	48.1	16.2	64.3	37.6	4.0	41.7	45.4	31.5	76.9
WCCC	47.2	145	60.7	46.9	10.9	57.7	58.1	34.0	92.1
LSD(0.05)	11.5	17.5	26.3	18.6	13.7	29.8	13.7	24.2	32.4
<i>P</i> -value									
Till (T)	0.064	0.905	0.737	0.647	0.110	0.329	0.014	0.074	0.583
Crop (C)	0.857	0.003	0.042	0.218	<0.001	0.007	0.021	<0.001	<0.001
T × C	0.540	0.908	0.796	0.421	0.217	0.407	0.189	0.085	0.102

CK refers to no cover crop control + 200 kg ha⁻¹ fertilizer N addition. HV=hairy vetch + 100 kg N ha⁻¹ addition, RC=red clover + 100 kg N ha⁻¹ addition, WCCC = white-crimson clover mix + 100 kg N ha⁻¹ addition. 2020 NO₃N was 44.8 mg N kg⁻¹ in CT versus 60.0 mg N kg⁻¹ and 64.7 mg N kg⁻¹ in NT and ST, respectively. LSD (0.05) = Protected Fisher's Least Significant Difference in a *P* value of 0.05.

TABLE 4 SPAD-502 chlorophyll meter readings for corn at the R1 growth stage with respect to cover crop species and termination-tillage method in 2018, 2019 and 2020.

	Chlorophyll reading (adimensional units)		
	2018	2019	2020
Cover Crop			
CK	55.0	51.8	58.8
HV	53.8	50.1	55.8
RC	54.9	49.2	55.1
WCCC	53.8	47.9	55.4
LSD(0.05)	1.9	2.9	1.9
Termination-Tillage Method			
CT	55.5	49.4	57.4
NT	53.9	50.8	56.0
ST	53.6	48.7	55.3
LSD(0.05)	1.6	2.4	1.7
<i>P</i> -value			
Till (T)	0.050	0.272	0.045
Crop (C)	0.419	0.023	0.002
T × C	0.765	0.217	0.885

CK = no cover crop + 200 kg N ha⁻¹ addition, HV = hairy vetch + 100 kg N ha⁻¹ addition, RC = red clover + 100 kg N ha⁻¹ addition, WCCC = white-crimson clover mix + 100 kg N ha⁻¹ addition. CT = cover crop termination by fall moldboard plow-down, then spring corn planting; NT = cover crop termination by spring herbicide spray-down and no-till corn planting; ST = cover crop termination by spring herbicide spray-down, then strip tillage and corn planting. Chlorophyll meter reading > 55 indicates an N sufficiency. LSD (0.05) = Protected Fisher's Least Significant Difference in a *P* value of 0.05.

cover crops (55.1–55.8). The SPAD readings were greater in 2018 and 2020 than in 2019.

3.5 Effects of cover crop species, termination-tillage method, and nitrogen addition on corn grain yields

Cover crop species, termination-tillage method and their interactions significantly impacted corn grain yields (Table 5). Corn grain yields from the no cover crop controls were not different among tillage-termination method in all three years, whereas grain yields in the cover crop treatments were often affected by an interaction of cover crop and tillage-termination method. Corn yields were CT > NT = ST for hairy vetch in 2018 and 2019, CT > ST > NT for red clover in 2018 and CT > NT for red clover in 2019, and CT > ST > NT for white-crimson clover mix in 2018 and 2019. In 2020, corn yields were ST > CT for hairy vetch, CT = ST > NT for red clover, and CT > NT for white-crimson clover mix. HV had similar corn yields as other cover crops under CT and ST but HV had greater yields than RC and WCCC under NT in 2018. In 2019, corn yields were CK > RC for fall plow-down, CK > HV = RC > WCCC for spring no-tillage, and CK > RC > WCCC for spring strip tillage. And in 2020, corn grain yields were CK > cover crops for fall plow-down, CK = HV > WCCC > RC for spring no-tillage, and CK = HV > RC = WCCC for spring strip tillage.

Year-to-year variation in corn grain yields reflected changes in weather and cover crop biomass (Tables S1, 2). While 2018 and 2020 received even and timely precipitation, corn planting was delayed substantially in 2019 (June 24, 2019), relative to the other two years (May 31 2018 and June 11 2020), as 2019 had the wettest spring in several decades for southwestern Ontario. Averaged over three years and termination-tillage method, corn grain yield was greatest for CK (11.7 Mg ha⁻¹), then corn after HV (10.5 Mg ha⁻¹), then corn after RC (9.4 Mg ha⁻¹), and then corn after WCCC (9.2 Mg ha⁻¹) (Table 5). Averaged over three years and cover crop species, corn grain yields were 11.2 Mg ha⁻¹ for CT > 10.3 Mg ha⁻¹ for ST > 9.2 Mg ha⁻¹ for NT (Table 5). Hence, cover crop termination by fall moldboard plowing generated the largest corn grain yield, and spring ST was better than NT. The 2020 corn grain yield in HV-ST (13.4 Mg ha⁻¹) was effectively equivalent to CK-ST (13.7 Mg ha⁻¹). The poorest corn yield occurred under WCCC-NT in 2019 (2.4 Mg ha⁻¹, Table 5), which were attributed to inadequate spring herbicide termination of white clover in that year.

3.6 Apparent fertilizer N replacement value, FNRV_A

There was a significant interaction between crop and tillage-termination method for FNRV_A in each year, and

FNRV_A varied widely from 35 to 92 kg N ha⁻¹ in 2018, from 28 to 81 kg N ha⁻¹ in 2019, and from 12 to 103 kg N ha⁻¹ in 2020 (Table 6). In 2018, FNRV_A was similar among cover crops for CT and ST, but FNRV_A was greater for HV than RC and WCCC under NT. In 2019, FNRV_A was similar among cover crop species for CT and NT, however, FNRV_A was greater for RC than WCCC under ST. In 2020, FNRV_A was similar among cover crop species for CT, but FNRV_A was greater for HV than RC and WCCC under NT and ST. Year 2019 produced the smallest mean FNRV_A (53 kg N ha⁻¹) compared to 2018 (69 kg N ha⁻¹) and 2020 (73 kg N ha⁻¹). The small N credit in 2019 was likely caused by low grain yields in that year due to late planting, poor corn stands and inadequate herbicide termination of white clover (Table 5; Figure 2). High FNRV_A occurred for HV in 2020 under all three termination-tillage treatments (85 – 103 kg N ha⁻¹); however, the FNRV_A values were lower for RC and WCCC under NT and ST, ranging from 12 to 73 kg N ha⁻¹. The three-year average FNRV_A was greatest for CT (83 kg N ha⁻¹), followed by ST (63 kg N ha⁻¹), and then NT (48 kg N ha⁻¹); and it was greatest for HV (75 kg N ha⁻¹), followed by WCCC (62 kg N ha⁻¹), and then RC (58 kg N ha⁻¹).

4 Discussion

Total global N fixation is on the order of 1.75 x 10¹¹ kg N yr⁻¹, with about half (i.e. ≈ 8 x 10¹⁰ kg N yr⁻¹ or 20–200 kg N ha⁻¹ yr⁻¹) fixed biologically by leguminous plants, and the rest fixed by industrial activity including fertilizer production (46). Although farmers still predominantly use synthetically-produced N fertilizer, biologically fixed N from legume crops is being increasingly recognized as a viable N source for crop production. The ability of legume plants to fix N from the atmosphere varies with plant species, growth stage and dry matter content of plant biomass (47).

In this study, legume C:N ratios ranged from 12.3 to 16.8, which are slightly higher than those for clovers in a study conducted ~90 km northeast of our study site (9.4 to 14.2, 24), but comparable with the C:N ratios obtained for clovers (13.8 to 15.6) and HV (13.2 to 13.9) in a study conducted on a sandy loam soil about 30 km south of our study site (35). The variability in C:N ratios by crop species could be the result of differences in both development and relative plant component composition (i.e. leaves vs. stems vs. roots) at the time of sampling. Legumes with a lower C:N ratio will decompose faster and release N more quickly than legumes with higher C:N ratios (17). Therefore, terminated HV will usually release biomass N somewhat faster than terminated RC and WCCC. The amount of accumulated legume biomass can also be affected by sampling time, particularly during April and May when plant growth rate increases dramatically and often differs substantially among plant species (48). In this study, greater accumulated

TABLE 5 Corn grain yields (15.5% seed moisture content) for cover crop species and termination-tillage method (2018-2020).

	Termination-Tillage Method			LSD(0.05)
	CT	NT	ST	
	----- Mg ha ⁻¹ -----			
2018				
CK (no cover crop)	12.4	11.7	11.2	0.7
Hairy vetch	11.7	10.8	10.5	0.8
Red clover	11.9	8.4	10.5	1.2
White-Crimson clover mix	11.9	8.4	10.2	0.9
LSD(0.05)	0.7	1.1	0.9	10.8
2019				
CK (no cover crop)	10.2	10.0	9.6	0.8
Hairy vetch	9.2	7.4	7.0	1.4
Red clover	8.3	7.1	7.8	0.9
White-Crimson clover mix	9.2	2.4 ^a	6.5	1.2
LSD(0.05)	1.4	0.9	0.9	7.9
2020				
CK (no cover crop)	13.2	13.4	13.7	0.6
Hairy vetch	12.2	12.7	13.4	1.0
Red clover	12.2	7.4	11.2	1.9
White-Crimson clover mix	12.2	10.2	11.4	1.3
LSD(0.05)	1.0	1.4	0.8	11.9
3-yr average				
CK (no cover crop)	11.9	11.7	11.5	0.9
Hairy vetch	11.0	10.3	10.3	1.5
Red clover	10.8	7.6	9.8	1.1
White-Crimson clover mix	11.1	7.0	9.4	1.7
LSD(0.05)	1.1	1.4	1.3	
P-value				
	2018	2019	2020	3-year mean
Tillage (T)	<0.001	<0.001	<0.001	<0.001
Crop (C)	<0.001	<0.001	<0.001	<0.001
T × C	0.002	0.053	<0.001	0.062
CK = no cover crop + 200 kg N ha ⁻¹ addition, HV = hairy vetch + 100 kg N ha ⁻¹ addition, RC = red clover + 100 kg N ha ⁻¹ addition, WCCC = white-crimson clover mix + 100 kg N ha ⁻¹ addition. CT = cover crop termination by fall moldboard plow-down, then spring corn planting; NT = cover crop termination by spring herbicide spray-down and no-till corn planting; ST = cover crop termination by spring herbicide spray-down, then strip tillage and corn planting. ^a In 2019, apparent herbicide failure on NT-WCCC occurred, which caused significant corn yield loss. LSD (0.05) = Protected Fisher's Least Significant Difference in a P value of 0.05.				

biomass and biomass N in 2020 is likely due to the fact that biomass sample collection in that year occurred 3 weeks later than in 2018, and one week later than in 2019 (Table 1). For example, two weeks additional growth between late April and

early May increased HV biomass by 61% in North Carolina (13) and by 35% in Maryland (23).

Red clover is a long-established cover crop in southwestern Ontario, but summer-seeding into winter wheat stubble is

TABLE 6 Apparent fertilizer nitrogen replacement value, $FNRV_A$ (Eq. 1), to corn for different cover crop species and termination-tillage methods.

Year	Cover Crop Species	Termination-Tillage Method			LSD(0.05)
		CT	NT	ST	
		----- kg N ha ⁻¹ -----			
2018	Hairy vetch	89.3	74.9	69.0	14.4
	Red clover	92.1	35.4	69.5	19.5
	White-Crimson clover mix	91.6	36.2	65.2	15.4
	LSD(0.05)	12.5	17.9	10.9	
2019	Hairy vetch	80.5	44.2	37.2	28.1
	Red clover	61.8	39.8	53.5	15.3
	White-Crimson clover mix	80.3	- ^a	28.1	26.0
	LSD(0.05)	29.4	18.7	20.7	
2020	Hairy vetch	85.2	92.5	103	18.7
	Red clover	85.3	11.9	69.5	30.0
	White-Crimson clover mix	84.5	54.4	72.9	20.6
	LSD(0.05)	22.1	35.1	14.2	
3-yr average	Hairy vetch	85.0	70.5	69.7	20.2
	Red clover	79.7	29.0	64.2	12.8
	White-Crimson clover mix	85.5	45.3	55.4	16.7
	LSD(0.05)	13.8	16.4	19.3	
		<i>P</i> -value			
		2018	2019	2020	3-yr mean
Tillage (T)		<0.001	<0.001	<0.001	<0.001
Crop (C)		0.048	0.830	<0.001	0.002
T × C		0.016	0.104	<0.001	0.010

CK = no cover crop + 200 kg N ha⁻¹ addition, HV = hairy vetch + 100 kg N ha⁻¹ addition, RC = red clover + 100 kg N ha⁻¹ addition, WCCC = white-crimson clover mix + 100 kg N ha⁻¹ addition. CT = cover crop termination by fall moldboard plow-down, then spring corn planting; NT = cover crop termination by spring herbicide spray-down and no-till corn planting; ST = cover crop termination by spring herbicide spray-down, then strip tillage and corn planting. ^a A negative N credit was achieved due to very low corn yield so it was not include in ANOVA. LSD (0.05) = Protected Fisher's Least Significant Difference in a *P* value of 0.05.

extremely rare compared to traditional frost-seeding (e.g. 49, 50). Coombs et al. (24) found less RC biomass accumulation for late May to mid-July planting relative to mid-March to mid-April planting, which they attributed to length of available growing period. Yang et al. (35) found less biomass and biomass N by freeze-up for summer-seeded RC than HV or crimson clover. The spring and fall biomass for RC in this study (5.8 and 5.0 Mg ha⁻¹, respectively) were in the range found for an organic crop production trial on a sandy loam about 30 km south (4.1 and 6.8 Mg ha⁻¹, respectively; 35), but were greater than the amounts found for another sandy loam about 90 km northeast (0.7 and 3.1 Mg ha⁻¹, respectively; 24), and also greater than “representative” values (0.7 and 4.2 Mg ha⁻¹) reported for temperate climates (10, 51, 52). Coombs et al. (24) noted that

when RC was allowed to over-winter, biomass increased relative to fall biomass for Ontario (49, 51, 53), Ohio and Michigan (45, 54). Over-winter increases in RC biomass occurred in this study, with an average fall biomass of 5.0 Mg ha⁻¹ increasing to 5.9 Mg ha⁻¹ by the following spring.

Hairy vetch and crimson clover are popular N sources for grain corn in the US (e.g. 11, 55). Hairy vetch is also used in Ontario because of its cold tolerance (35, 56, 57) whereas crimson clover is rarely used. The nitrogen content in HV above-ground biomass ranges from 75 kg N ha⁻¹ to 288 kg N ha⁻¹ at various sites in Alabama, Georgia, Kentucky, and North Carolina (11, 56, 58). Van Eerd (57) obtained relatively low biomass N (41 – 139 kg N ha⁻¹) by freeze-up for HV planted in early August in southern Ontario, as well as little re-growth in

the following spring. This differs from our results, and may reflect our milder climate and/or soil differences. This study showed that HV and crimson clover performed well for accumulating above-ground biomass and biomass N in both the fall and the following spring.

Corn leaf chlorophyll level has been used for determining plant N status when a reference N level is available and all factors other than N are constant (59). SPAD-502 chlorophyll readings from the well-fertilized controls (200 kg N ha^{-1}) were used as the reference N level (60–63), and N deficiency in the cover crop treatments was deemed to occur when their SPAD readings fell below 95% of the reference. This occurred for WCCC in 2019 and for RC and WCCC in 2020. If, however, the 93% critical SPAD level of Piekielek et al. (61) is used, then none of the treatments were N deficient. The SPAD readings closely followed measured cover crop biomass N (Table 2), but showed no clear impacts on corn grain yields (Table 5). The SPAD readings at corn R1 growth stage were inconsistent with soil mineral N measured one month after corn planting (Table 3), indicating sustained release of N from legume decomposition (58, 64–66). The poor match between soil N or SPAD readings and corn yields indicates that soil N may not be a limiting factor for corn yield in this study. It appears instead that the primary factor affecting corn grain yields was corn stand number, which showed a highly significant positive correlation with corn grain yields (Figure S3).

Although results were not consistent among cover crops and years, this study never-the-less supports the general consensus that summer-seeding legume cover crops after winter wheat harvest can contribute substantial N to a succeeding corn crop in southwestern Ontario (35, 49, 57) and in the northern United States (58, 67, 68). The FNRV_A in this study were comparable to the 65 to 130 kg N ha^{-1} from legume cover crops found in a Maryland study (69). The highest FNRV_A (100 kg N ha^{-1}) in our study is also equivalent to that obtained in an Ontario study where oat inter-seeded with red clover provided N to a following corn crop (70). In Ohio, Ngalla and Eckert (71) compared frost-seeding red clover into winter wheat with July seeding of red clover after wheat harvest. They found that frost-seeded red clover contributed 56 to 67 kg N ha^{-1} to the following corn, but summer-seeded red clover provided effectively no N benefit because of insufficient biomass accumulation.

Partial replacement of synthetic fertilizer N with legume cover crop N often caused a substantial grain yield penalty for no-tillage, especially for RC-NT and WCCC-NT. This occurred because surface mulches, such as cover crop residues, often delay dry-down and warming of Brookston clay loam soil in southern Ontario, and this in turn reduces corn germination and early growth. Hence, RC and WCCC cover crops are not recommended for fine-textured soils under NT in southern Ontario. On the other hand, NT after HV often produced acceptable plant stands, and the higher N benefit from HV contributed to acceptable corn yields. Higher N contribution

from HV was also found in Brazil, and was deemed a viable strategy for boosting corn yield under NT (72). Vaughan et al. (73) found that combining HV with NT increased the yield potential of both monoculture and rotation corn in North Carolina.

In summary, the legume cover crops, hairy vetch, red clover, crimson clover and white-crimson clover mix, could all enhance the traditional soybean – winter wheat – corn rotation in southwestern Ontario when the cover crops are summer-seeded after wheat harvest. Hairy vetch was superior to the other cover crops, however, as it generated larger amounts of biomass and accumulated greater amounts of biomass nitrogen. Further, hairy vetch is easily terminated in the fall or spring, it allows good corn emergence, and it can provide the equivalent of 85 kg N ha^{-1} of fertilizer to the corn crop. Averaged over three years and three termination-tillage systems (i.e. fall moldboard plow-down with conventional tillage, spring herbicide spray-down with strip-tillage, spring herbicide spray-down with no-tillage), corn yields following hairy vetch were 13% greater than corn yields following red clover and 15% greater than corn yields following a white-crimson clover mix. If fall termination of the cover crop is desired, summer-seeding hairy vetch or white and crimson clovers are recommended for fine-textured soils in southwestern Ontario because they accumulate more biomass relative to red clover. If strong spring re-growth is desired, hairy vetch is more desirable than red, white and crimson clovers because clovers tend to be more difficult to terminate with herbicides in the spring, and they can reduce grain yields by delaying corn emergence and reducing final corn stands.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Author contributions

XY and WR conceptualized the work; CD managed soil and crop N analysis; M-AR collected and analysed the soil and plant samples. XY planned the experiment and wrote the first draft. All authors contributed to manuscript editing and approved the final submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsoil.2022.1082940/full#supplementary-material>

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