INTERSEEDED COVER CROPS IN ONTARIO GRAIN CORN SYSTEMS: POTENTIAL IMPLICATIONS FOR CORN NITROGEN UPTAKE, SOIL HEALTH, AND RESIDUE DECOMPOSITION

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ABSTRACT

Interseeded Cover Crops in Ontario Grain Corn Systems: Potential Implications for Corn Nitrogen Uptake, Soil Health, and Residue Decomposition

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Ontario grain corn is highly valuable, accounting for 60% of Canada's total corn output. Grain producers are increasingly interested in including cover crops (CCs) in their cropping systems, but they have concerns regarding successful CC establishment and potential adverse competitive effects on corn yield and nutrient status. One option to improve the success of CC establishment is the interseeding in corn at the V4 -V6 stages. Interseeding improves the chances of good CC establishment, with potential benefits for soil health, weed control, and plant productivity. This thesis research was conducted to evaluate the short-term effectiveness of interseeding annual ryegrass (AR), red clover (RC), and their mixture (MIX) in grain corn at three locations in central and southwestern Ontario. Cover crop and corn yields, and their nitrogen (N) uptake, residual soil N, soil biological parameters, weed biomass, and residue decomposition rates were measured. CC biomass was highly variable (range: 0 - 1.6 Mg ha^{-1}), influenced by climatic conditions, location, and CC type. Total carbon (C) and N contributions from CCs were similarly influenced by site-year and CC type. Regression analyses showed significant influence of corn biomass on CC establishment. Red clover had a significantly lower C/N ratio (11.8) than AR (18.2) and MIX (15.6). Strikingly, the amount of CC biomass accumulated in early spring reduced weeds by 50%. Moreover, CCs did not reduce corn grain or stover yield, nor N uptake, and soil mineral N in either fall or spring. Soil metabolic activity measured by BIOLOG Ecoplates was significantly greater in plots with AR than RC, MIX or NOCC. Soil biological parameters showed no CC effect. Results of residue decomposition i.e., C and N mineralization showed negligible CC residue effects on corn stover decomposition or N immobilization. The findings from this research suggest the need for assessing a more diverse range of CCs over longer durations to establish more specific CC niches for improving soil health in Ontario corn systems.

Key words: annual ryegrass, cover crops, CLPP, grain corn, interseeding, nitrogen uptake, red clover, residue decomposition, soil health

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1. INTRODUCTION AND THESIS OUTLINE

In Ontario, grain producers are increasingly interested in including cover crops (CCs) in their cropping systems, but insecurities about successful cover crop establishment, biomass production, and realization of significant benefits are some of the challenging issues that have led to the slow adoption of CCs in the province. Almost 50% of Canadian grain corn is grown in either corn-soybean-wheat or corn-soybean rotations. In these rotation systems, soybean follows corn and there is not much window for the inclusion of CCs. Intersowing CCs at the V4-V6 stages potentially improves the chances of good establishment, hence the potential benefits from CC biomass. Corn-soybean rotations stand to benefit from successful inclusion of CCs, and benefits include enhance crop diversity, maintenance of soil cover between cash crop cycles, weed suppression, erosion control, and soil organic matter (SOM) addition. The interseeding of CCs into grain corn is not a new concept in Ontario, but the practice has not yet been adopted by producers due to lack of knowledge on the extent of benefits and risks. There is interest in evaluating this practice as it may be the most promising option for successful inclusion of CCs in Ontario corn-based rotations.

This PhD thesis research assesses the potential for successfully establishing annual ryegrass and RC in standing corn. Previous studies have shown potential for success with no negative impacts on corn yield or quality when CCs are interseeded into standing corn (Abdin et al., 1998; Belfry and Van Eerd, 2016; Wall et al., 1991). Based on insights from the studies that have been conducted with different CCs in ON, annual ryegrass and RC were selected as the test crops for this research. Previous studies with interseeded CCs have focused on establishment success, corn yield, and nitrogen dynamics, but none have assessed the responses of soil health parameters.

In early May 2015, field trials were established at three locations in southern Ontario (Elora, Ridgetown, and Peterborough). At each site, AR, RC, and their mixture (MIX) were interseeded into corn at the V4 – V6 corn stage. Establishment was monitored to quantify treatment size for soil health assessment. Agronomic and soil responses were evaluated over two seasons, 2015 - 2017. Chapters 2 and 3 of this thesis are based on results from the field experiment.

In Chapter 2, corn yield, N uptake and concentration in tissues, as well as residual SMN are assessed. The responses of dynamic soil health parameters are evaluated in Chapter 3. Soil health parameters were selected based on their potential to quickly respond to short-term changes in management. To complement the field experiment, an incubation study was conducted (Chapter 4) to evaluate the potential rates of corn residue decomposition (C and N mineralization) in the presence of CC residues. The final chapter of this thesis is a general discussion which includes a summary of findings from the field and incubation experiments and recommendations for future studies or decision making with regards to annual ryegrass and RC inclusion in grain corn-based rotations in Ontario.

2. LITERATURE REVIEW: PERSPECTIVES ON OPPORTUNITIES FOR COVER CROP ADOPTION IN CANADA

2.1. Overview of Canadian Cropping Systems

The agriculture and agri-food system in Canada is valued at $143.1 billion (over 7% of the national annual GDP), and supplies a wide range of food and commodities to both domestic and global markets (AAFC, 2013). According to Agriculture and Agri-food Canada (AAFC), continued growth and intensification of production, timely adaptation to global market demands, and advances in innovative technologies over the past century have made Canada the 5th largest global exporter of agricultural produce (AAFC, 2013; Statistics Canada, 2017a). Of Canada's 909 million ha total land area, only 7% (64 million ha) is used for agriculture, with 98% of the cultivated cropland in five of the ten provinces: Saskatchewan, 43.7%; Alberta, 27.2%; Manitoba, 12.4%; Ontario, 9.47%; and Quebec, 4.74% (Statistics Canada, 2017a). Within each of these five provinces, 60 – 77% of the arable land is seeded to hay and field crops, the rest being under woodlands, summer-fallow, or natural pasture [Figure 2.1; Statistics Canada (2017b)]. Although only a small portion of the total land area is cultivated, intensive tillage and use of chemical inputs are predominant, particularly in corn production (Deen et al., 2013).

The continued growth and intensification of Canadian agricultural systems has resulted in vast areas seeded to a few high-value, input-intensive crops such as soybean, canola, spring wheat, and corn (Deen et al., 2013; McRae et al., 2000; Statistics Canada, 2017a). Within each region, there is also a decreasing trend in the number of agricultural operations, consequently leading to a corresponding increase in the average size and capital-intensity of the resultant operations (Statistics Canada, 2017a). Overall, the diversity of crops currently dominating the agricultural landscapes of the top five provinces (SK, AB, MB, ON, and QC) has decreased, and 55 - 76% of provincial croplands are used to produce only three crops (Table 2.1). While the economic milestones of the Canadian agricultural system are to be celebrated, there is a growing concern over increased risks of soil and environmental degradation associated with agricultural intensification (Cambareri and Grant-Young, 2018; Struik et al., 2014). For production systems to be sustainable, there is need for balanced prioritization of productivity, quality, economic gains, and environmental stewardship.

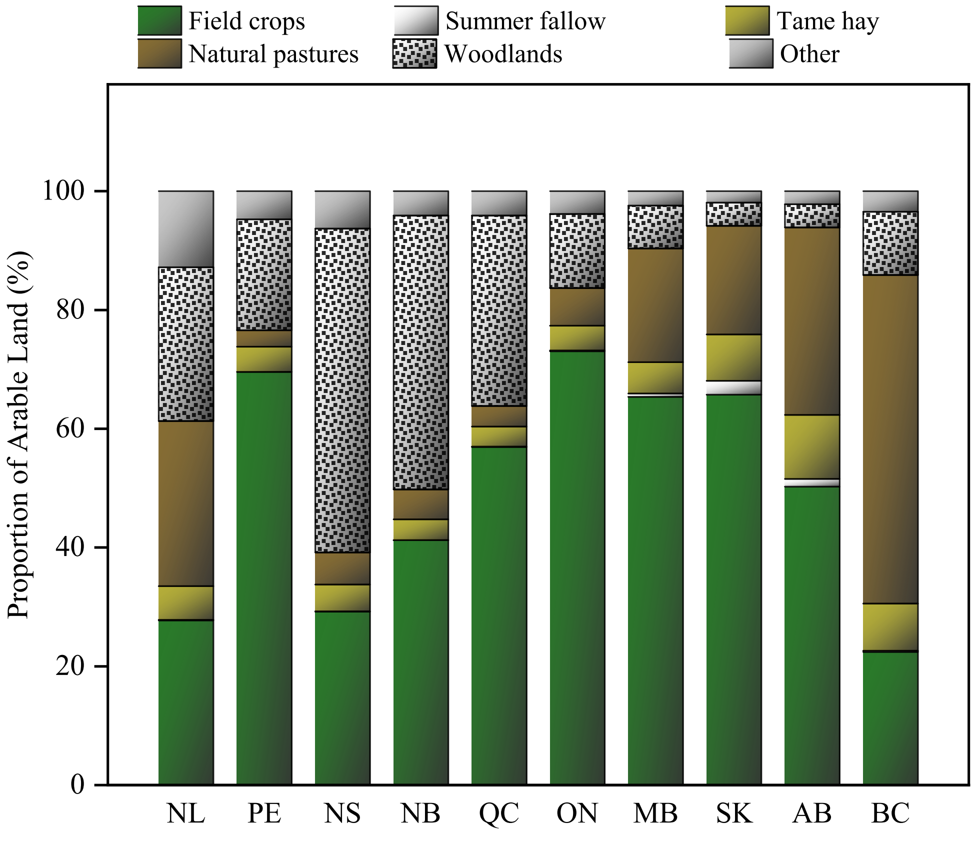


Figure 2.1. Land-use allocation for arable land in Canadian provinces as of 2016. *Data from Statistics Canada (2017b). Woodlands include areas with Christmas trees, woodlands, and wetlands.*

Table 2.1. The portion of arable area under hay and field crops and the five most common crops for Canadian provinces in 2016. *Data: Statistics Canada (2017d)*

Province	**Total Cropped Area (x1000 ha)**	**Major crops ranked by size of cultivated area (x1000 ha)**				
		1st	**2nd**	**3rd**	**4th**	**5th**
CANADA ***193,492†***	**37,504**	Canola *8,339‡*	Spring wheat *6,351*	Alfalfa mixes *3,754*	Barley *2,710*	Durum wheat *2,454*
SK ***34,523***	16,383	Canola *4480*	Spring wheat *2708*	Lentils *2067*	Durum wheat *2003*	Alfalfa mixes *1158*
AB ***40,638***	10,211	Canola *2495*	Spring wheat *2318*	Barley *1382*	Alfalfa mixes *1237*	Dry field peas *773*
MB ***14,791***	4,660	Canola *1295*	Spring wheat *1144*	Soybeans *666*	Alfalfa mixes *429*	Fodder/hay *184*
ON ***49,600***	3,553	Soybeans *1126*	Grain corn *875*	Alfalfa mixes *453*	Winter wheat *437*	Silage corn *120*
QC ***28,919***	1,779	Fodder/hay *422*	Grain corn *397*	Soybeans *352*	Alfalfa mixes *235*	Oats *84.4*
BC ***19,759***	544	Alfalfa mixes *198*	Fodder/hay *150*	Spring wheat *40.8*	Canola *38.5*	Oats *27.3*
PE ***1,353***	154	Potatoes *33.7*	Fodder/hay *33.6*	Barley *24.9*	Soybeans *18.2*	Alfalfa mixes *15.1*
NB ***2,611***	120	Fodder/hay *51.5*	Potatoes *18.7*	Alfalfa mixes *13.5*	Oats *10.2*	Barley *6.9*
NS ***3,478***	80.3	Fodder/hay *45.8*	Alfalfa mixes *13.5*	Grain corn *8.11*	Silage corn *4.63*	Winter wheat *3.0*
NL ***407***	6.95	Fodder/hay *4.71*	Alfalfa mixes *1.57*	Silage corn *0.21*	Potatoes *0.15*	Spring wheat *0.10*

† Values under each province represent the total number of farms
‡ Values under each crop represent the total provincial land area seeded to that crop

2.2. Agriculture and Ecosystem Services

Agricultural landscapes provide a plethora of ecosystem services (ESs), including the provision of food and fibre, regulation of soil and water quality, nutrient cycling, C sequestration, and supporting biodiversity (Hoffmann et al., 2018; Kragt and Robertson, 2014; Power, 2010). The extent to which agriculture consumes, supports, or weakens the provision of these ESs is greatly influenced by farm management practices. For instance, the choice and range of crops grown (species diversity) as well as how they are grown (e.g., tillage practices or use of mineral and organic fertilizers, herbicides, and pesticides) determine the extent to which farms provide food (Bommarco et al., 2018), support insect and wildlife diversity (Emmerson et al., 2016), sequester C (Lal, 2019), or enhance/degrade soil (Chahal and Van Eerd, 2018b; Govers et al., 2017; Olson et al., 2017), air (Huddell et al., 2020) and water quality (Hoffmann et al., 2018). As farmers intensify their operations and dedicate more land to increasingly narrower ranges of more economically viable crops, the capacity for agroecosystems to offer ESs is compromised (de Graaff et al., 2019; McRae et al., 2000; Tilman, 2020).

Decreases in ESs from agroecosystems are accompanied by increases in environmental risks to farms and their surrounding landscapes. Risks include increased soil erosion (Olson et al., 2017) and water contamination (Liu et al., 2018; Zhang et al., 2007), declining soil organic carbon (SOC) levels (Fox et al., 1991; McRae et al., 2000; Olson et al., 2017), or loss of biodiversity (Emmerson et al., 2016). It is therefore imperative that agricultural systems, especially those with intensive operations, promote practices that enhance ESs. Key practices that promote provision of ESs from cropping systems include the diversification of crop rotations and optimal soil cover through intercropping [simultaneous planting of two or more crops (Tilman, 2020)] or cover cropping [planting non-commodity crops to keep the soil covered during fallow periods (Daryanto et al., 2018; Finney and Kaye, 2017)]. Research has shown that high crop diversity promotes the sustainability of agroecosystems through increased yields and yield stability (Ponisio and Ehrlich, 2016), promoting soil microbial biodiversity (King and Hofmockel, 2017; Lange et al., 2015), enhancing weed and pest suppression (Isbell et al., 2017; Letourneau et al., 2011), and improving soil health (Bünemann et al., 2018; Karlen et al., 2019)]. All these benefits ultimately lead to greater balance between environmental and agronomic objectives (Marr et al., 2016), enhance agroecosystem resilience in the face of environmental disturbances (Li et al., 2019; Lin, 2011; McDaniel et al., 2014b), and climate change (Paustian et al., 2016).

2.3. Soil Health

Healthy agricultural soils are those that can sufficiently support food and fibre production while supplying other ESs to maintain the quality of human life while conserving biodiversity (Kibblewhite et al., 2008; Rinot et al., 2019). Such soils are also expected to be resilient to extreme or erratic weather conditions, having greater capacity to maintain crop productivity in the face of climate change (Congreves et al., 2015). By definition, soil health is "the ability of soil to function as a vital living system that can sustain biological productivity, promote environmental quality, and maintain plant, animal and human health" (Karlen et al., 2019). Some scholars have used the terms 'soil health' and 'soil quality' interchangeably [e.g.,(Haney et al., 2018) Bünemann et al. (2018); Idowu et al. (2009); Karlen et al. (2017)]. However, there is a school of thought that argues that the two concepts are fundamentally different because the concept of soil health considers soil as a living and dynamic system (Cardoso et al., 2013; Doran et al., 1997; Doran and Zeiss, 2000), whereas the concept of soil quality is tied only to the intended functions of a soil (e.g., food and fiber production, recreation, and waste recycling) (Karlen et al., 2003;

Karlen et al., 1997). The two concepts describe the capacity of soil to function within boundaries set by inherent properties, e.g., parent material, climate, biota, and topography. Those that prefer not to make a distinction argue that soil health is equivalent to ‘dynamic soil quality’ because it places emphasis on the biotic component and biological processes of the soil, and greatly overlaps with the same idea as soil quality depending on the context (Bünemann et al., 2018). Karlen et al. (2019) elucidate the evolution of the two concepts, as well as past and ongoing debate about their appropriate uses. In this thesis, I focus on soil health as it relates to the soil attributes that are responsive to or can be maintained, promoted, or dimished by management choices.

Quantifying soil health is highly complex and involves the careful selection of a suite of pysical, chemical and biological soil parameters (indicators) on the basis of the inherent capabilities of each specific soil (Bruns, 2014). There are numerous soil health indicators ranging form physical, chemical, and biological soil attributes, and these have been extensively discussed in literature (Moebius-Clune, 2016; Nunes et al., 2020; Rinot et al., 2019; Roper et al., 2017; Trivedi et al., 2016). Physical indicators include porosity, aggregate stability, water infiltration, bulk density, and soil available water. Chemical indicators include pH, electrical conductivity (EC), leachable salts, cation exchange capacity (CEC) and plant available nutrients. Lastly, biological indicators include SOM, respiration, active or labile carbon, soil microbial biomass C and N, microbial community structure and diversity, potentially mineralizable N (PMN) and soil enzyme activities. There is no concensus among scientists with regards to the benchmarks or minimum datasets required for comprehensive soil health assessments (Haney et al., 2018).

To date, a few assessment tools based on standardized protocols are available for researchers, land managers, and consultants e.g., the Cornell Soil Health Test (Gugino et al., 2009; Idowu et al, 2008) or the Ontario Soil Health Assessment (Congreves et al, 2015). In these hollistic tests, effective indicators/response variables that meet the criterion as elucidated by Doran and Zeiss (2000) are used. Effective indicators are those that are sensitive to changes in management, well correlated with beneficial soil functions, useful for explaining ecosystyem processes, easily understable to land managers, and simple and inexpensive to measure (Doran and Zeiss, 2000). It is not always feasible to assess all attributes that satisfy a minimum dataset for soil health assessment, or to use standardized soil health assessment tools for every study, hence researchers often select response variables that are most appropriate to the treatments or management practices under assessment in their studies. In this thesis research, no particular soil health index was applied, but an extensive set of biological soil attributes with quick turnover times were assessed.

2.4. Cover Crops

Cover crops are non-commodity crops either interseeded into living cash crops or planted onto bare fields during fallow periods, primarily to maintain soil cover (Delgado et al., 2017; Wittwer et al., 2017). Some examples of common CC species are listed in Table 2.2., and these include cereal rye, *S. cereale*; hairy vetch, *V. villosa*; oilseed radish, *R. sativus*; annual ryegrass, *L. multiflorum*; crimson clover, *T. incarnatum*; and RC, *T. pratense*. Many agronomic and environmental benefits of CCs have been documented and are widely acknowledged by researchers, producers and other agricultural stakeholders

(Reeves, 2017; Sarrantonio and Gallandt, 2003; Schipanski et al., 2014). Benefits of CCs include soil protection from erosion through maintenance of soil cover (Blanco-Canqui et al., 2017) or improved soil aggregation and root architecture (Saleem et al., 2020); improvement of soil health by adding organic matter and extending carbon (C)-fixation periods (Ruis and Blanco-Canqui, 2017; Wittwer et al., 2017); pathogen (Latz et al., 2015) and weed suppression (Osipitan et al., 2018; Baraibar et al., 2018; O'Reilly et al., 2011; Teasdale et al,. 2007); and increased N supply (Coombs et al., 2017; Kaye et al., 2019; McKenna et al., 2018). The benefits of CCs are attributed to successful establishment and biomass accumulation (Barel et al., 2018; Finney et al., 2016; Florence et al., 2019). As CCs are inherently capable of simultaneously providing multiple ESs, producers potentially stand to benefit from additional benefits beyond their target objectives.

When successfully established, CCs can act as catch crops, capturing excess nutrients such as N and phosphorus (P) to minimize losses due to leaching and runoff (Blanco-Canqui et al., 2015; Kaye et al., 2019). Research has shown that more than 70% of soil NO_3-N could remain in the top 30 cm of the soil following corn harvest (Crandall et al., 2005). In their meta-analysis, Tonitto et al. (2006) reported that 40 to 70% of excess N can be captured by CCs. In addition to being highly efficient in reducing N leaching, non-legumes can also provide significant weed suppression benefits due to their substantially fast growth rates (Baraibar et al., 2018; Plaza-Bonilla et al., 2015; Tribouillois et al., 2016; White et al., 2017). On the other hand, leguminous CCs can be grown as green manures to provide N to concurrent (Dahlin and Stenberg, 2010; Rasmussen et al., 2013) and subsequent crops (Büchi et al., 2015; McKenna et al., 2018). The inclusion of leguminous CCs also enhances crop diversity in cereal-dominated rotations.

Cover crops from different plant families and varieties can be seeded in mixtures to increase chances of success and widen the range of benefits obtained from CC inclusion (Finney and Kaye, 2017; Finney et al., 2016; Nyfeler et al., 2011; Schipanski and Drinkwater, 2012). Cover crops grown in mixtures have potential to provide a safeguard when environmental conditions selectively fail one or more species in the mixture (Barel et al., 2018; Wortman et al., 2012). Relative to CC monocultures, the success of CC mixtures generally depends on the compatibility of the species in the mixture (minimal inter-specific competition) and the realization of benefits that are equal to or exceeding those of individual monocultures (Florence et al., 2019). Chapagain et al. (2020) provided a detailed stepwise procedure for selecting complementary CC mixtures based on a multifaceted criterion which includes grower objectives, cropping system compatibility, costs, planting equipment required, persistence/weediness, and potential net economic returns.

2.4.1. Cover Crop Adoption: Challenges and Opportunities

Despite all the potential benefits, CC inclusion also involves additional costs to producers as they must modify their operations and budgets to include CCs. Successful establishment, costs, and benefits of CCs vary both spatially and temporally (Appelgate et al., 2017; Blanco-Canqui et al., 2017), and are highly influenced by CC species or variety (Thiessen Martens et al., 2005; Tribouillois et al., 2015), climatic variability (Kim et al., 2020), and management. Practices such as planting time and termination and fertilization strategies (Ketterings et al., 2015; Murrell et al., 2017; Romdhane et al., 2019; Smith et al., 2014; Teixeira et al., 2016; White et al., 2017) can determine the extent to which CC benefits are realized by producers. Consequently, the extent of adoption and choices of CCs are fully dependent on farmers' objectives and CC compatibility with the overall management system (Hemkemeyer et al., 2017). The development of specific guidelines for local regions based on local research could go a long way in promoting CC inclusion. Whether grown in monocultures or mixtures, the inclusion of CCs in Canadian agricultural systems warrants robust promotion, especially in view of the increasing interest in, and the need for environmental conservation.

Current evidence suggests that overall, CCs can be managed profitably (Bergtold et al., 2017), and the direct and indirect benefits to farms and ecosystems far outweigh costs (Bergtold et al., 2017; Hayden et al., 2014; Sawadgo et al., 2019; Snapp et al., 2005). However, the successful inclusion of CCs depends on whether CC management strategies are designed to match the desired benefits, as well as to adapt to changing environmental conditions in a manner that minimizes risks. The window of CC establishment should ideally match the overall system for intended benefits to be realized. For instance, as Schipanski et al. (2014) observed, nutrient retention benefits primarily occur during CC growth, while weed suppression benefits occur during cash crop growth through a CC legacy effect, and soil C benefits accrue slowly over decades. So, if the goal is to capture excess nutrients, active CC growth should be synchronized with the periods of greatest risks of leaching, e.g., following cash crop harvest. Similarly, if building soil C is the objective, then the producer would need to grow high biomass CCs for extended periods of time.

The extent of adoption and continued use of CCs largely depends on the degree of expectation and realization of benefits, both in spatial and temporal scales, i.e., producers' capacity to assimilate the trade-offs between CC benefits, production costs, and management risks (Schipanski et al., 2014). Results from surveys in the US Corn Belt highlight that CC adoption is mostly hindered by negative perceptions associated with real and perceived risks of CC inclusion (Baumgart-Getz et al., 2012; Roesch-McNally et al., 2018a). The risks include: additional costs (Sawadgo et al., 2019); lack of financial incentives (Dunn et al., 2016) or access to information and trusted technical advisers (Eanes et al., 2017); inconsistencies in realization of immediate benefits; skepticism about yield reductions; challenges in successfully planting or terminating them when farmers do not have the proper equipment; limited establishment of decent stands before winter (Oliveira et al., 2019) and lack of interest and knowledge (Dunn et al., 2016; Mervin and McLarty, 2017; Snapp et al., 2005). Moreover, relaxed policies towards efforts to encourage adoption can also reduce CC adoption (Marr and Howley, 2018; Roesch-McNally et al., 2018c). Site-specific information on direct benefits of CCs could go a long way to address some of these barriers, encourage and support policy changes, and ultimately promote CC adoption.

Tonitto et al. (2006) provide a detailed assessment of how the interaction of climate and management factors influence the realization of CC benefits and potential drawbacks. Due to the erratic nature of most CC benefits and the time sensitivity of management windows, it is challenging to predict CC inclusion outcomes (Blanco-Canqui et al., 2015; Daryanto et al., 2018). For instance, numerous studies have shown minimal to no yield losses due to CC inclusion (Bich et al., 2014; Coombs et al., 2017; Gaudin et al., 2015; Gieske et al., 2016), while some studies have shown contradictory results that suggest potential yield losses under some conditions (Miguez and Bollero, 2005; Sharma and Banik, 2015; White et al., 2017). These different outcomes make it harder to convince those producers who are already sceptical about the benefits to adopt CC inclusion. It is therefore worthwhile to expand research so that knowledge gaps that exists about potential CC niches for specific regions can be filled. To promote CC adoption, there ought to be integrated efforts from scientists, policymakers, producers, and other agricultural stakeholders. Individual decisions taken at the farm/operation level are critical drivers for conservation goals at the landscape, regional or national scales. Therefore, supporting farmers with policy tools and scientific knowledge that promote the adoption of conservation practices is essential for achieving good environmental stewardship goals for the whole system.

2.4.2. Cover Crops in Canadian Agricultural Systems

Many strides have been made in the Canadian agricultural system with regards to the adoption of conservation practices. For instance, the number of bare-soil days significantly decreased between 1981 and 1996 due to the adoption of conservation tillage practices and significant reductions in summer-fallow practices across the prairie provinces (AB, SK, and MB), where ~46% of Canada's cropland lies [Figure 2.1; McRae et al. (2000); Statistics Canada (2017b)]. Although still limited, there have also been positive trends in adoption of CC and green manure practices [Figure 2.2; Statistics Canada (2017c)]. As of 2016, the highest number of farm operations with either green manures or CCs were in ON (42.5%) and QC (20.6%) (Figure 2.2). Conversely, less than 10% of farms practice either green manure incorporation or cover cropping in the Prairie provinces. Available data from Statistics Canada does not show the proportions of CC adoption based on cropping system (e.g., organic vs conventional) or farm sizes, but clearly suggests there is room for improvement overall. Opportunities for CC inclusion exist in Canadian field cropping systems, particularly in wide-row crops such as corn or potatoes, rotation systems that include winter wheat, or perennial systems such as vineyards and orchards.

In southern Ontario, the relative change in the proportion of farms reporting CC use between 2010 and 2015 increased in the range of 5 - 50% (Statistics Canada Agriculture Division, 2018). However, it has also been argued that the adoption of practices that promote crop diversity in Ontario has been limited to small-scale adoption, mostly in organic systems (Deen et al., 2013; Marr and Howley, 2018; Wayman et al., 2017). Also, uncertainties with regards to the risks of yield reduction pose the greatest threat to CC adoption in Canada (Martens et al., 2015). This suggests that there is great potential for larger scale adoption across the province, which can be enabled by significant shifts in policy and research objectives that enhance farmers' knowledge, confidence, and willingness to adopt CCs.

In 2017, the Ontario Ministry of Agriculture and Rural Affairs published the *Ontario Cover Crop Strategy*, which highlights that CC adoption in Ontario is limited by challenges of establishment, time and labor constraints, as well as finding the right cash and CC species and varieties for specific production systems and soil and climatic conditions (Mervin and McLarty, 2017). There is limited time to successfully establish CCs after cash crop harvest in the increasingly popular corn-soybean rotations. The growing season in Ontario is short and there are very limited time windows for CCs to establish substantially enough to produce significant biomass or other desired benefits when planted after corn or soybean harvest (Liebig et al., 2015). Cover crop inclusion in rotations involving winter grains, e.g., corn-soybean-winter wheat, is typically less challenging due to the longer time window of establishment between winter wheat harvest and the start of the frost period. One viable option for rotations that do not contain winter wheat is the interseeding of CCs into corn earlier in the spring. Corn offers the best opportunity for CC inclusion due to the wide row spaces (76-cm). Research has shown that spring-interseeding is particularly successful at the V5 stage when corn plants are well established and competition with the CC is less likely to be detrimental to yield (Belfry and Van Eerd, 2016; Bich et al., 2014; Brooker et al., 2020). There is immense need for more research in Ontario and across all agricultural regions of Canada to evaluate CC niches for each specific climatic region and management system.

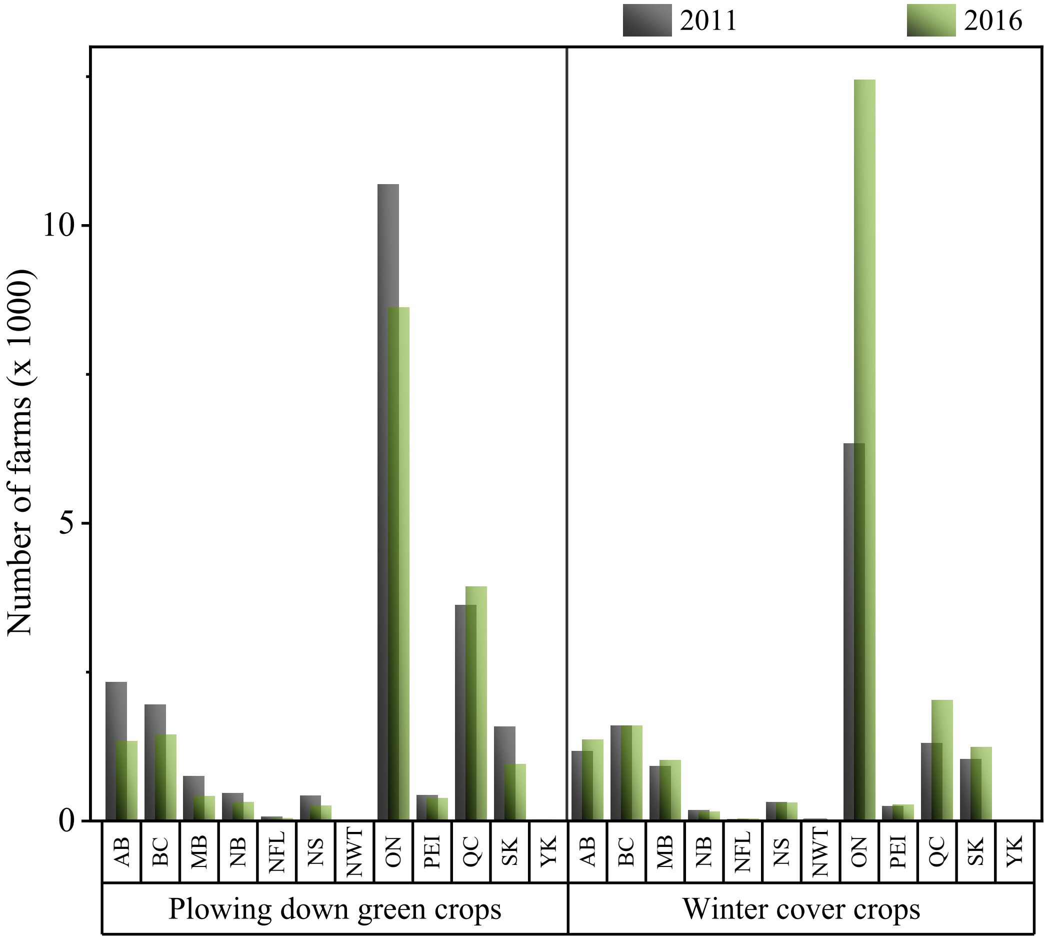


Figure 2.2. Use of winter cover crops and green manure on farms in Canadian provinces and territories in 2011 and 2016. The total number of farms for each province are given in Table 1. *Data source: Statistics Canada (2017c)*

2.5. Cover Crop Research in Canada

Numerous studies have assessed cover cropping options for North American cropping systems, mostly in the US (Appelgate et al., 2017; Blanco-Canqui, 2018; Daryanto et al., 2018; Delgado et al., 2007; Marcillo and Miguez, 2017; Schipanski et al., 2014), and relatively fewer studies in Canada (Table 2.2). Although there are vast amounts of general guidelines from studies conducted elsewhere, effective CC adoption requires more home-grown research efforts that can directly address the needs of Canadian stakeholders. To date, there has not been a synthesis of published studies to highlight the current scale and scope of research within Canada. It is therefore difficult to assess the areas or systems with the greatest potential for CC inclusion. Coordinated efforts to consolidate scientific data for Canadian regions and subregions may promote interest by farmers or potentially influence significant policy changes that promote widespread adoption in Canada.

A literature search on the Web of Science Core Collection with the key words “cover crop” or “winter cover crops” for Canada returned 152 peer-reviewed articles published in the past four decades (1981 – 2020). Of these, only 45 had CCs as the primary treatments being assessed (Table 2.2). Based on these available records, published CC research on field crops in Canada was very limited before 2010 (only 14 out of 45 studies). The past decade has seen steady growth in the number of published studies, and publications increased markedly in the past five years, with 62% of published studies between 2015 and 2020. Overall, there are more publications (23 out of 45) from Ontario compared to the rest of the provinces, mirroring the higher trend for CC adoption also recorded from the latest census (Figure 2.2). These corresponding trends are indicative of a strong positive link between CC research and adoption. The available studies have focused on a variety of objectives that address producer concerns including yield responses, weed and disease suppression, erosion control, N cycling and soil health. More than 50% of studies focused on CC effects on crop yields, while 49, 29, 13, and 4.4% assessed N supply, soil health, weed suppression, and erosion control, respectively. Table 2.2 shows the disproportionate distribution of research across Canadian provinces and highlights the need for more research focusing on different variables that showcase CC niches and benefits to promote interest, particularly for the major crops dominating the Canadian agricultural landscape.

2.6. Findings from Canadian Studies Involving Cover Crops

2.6.1. Cover Crop Establishment

Research in Canada shows variable success of CC establishment for different crops and regions, and the significance influence of climate, CC type and cropping systems (Ruis et al., 2019). Failure of CC establishment is common, especially if CC planting is done late in the fall (Li et al., 2015). Not all studies have reported cases of CC establishment failure, but research has shown that establishment can be limited in temperate regions such as those in Canada (Table 2.2). Available data from studies involving winter rye interseeded in grain corn in ON showed 2.7 – 4.5 Mg ha^{-1} and 5.4 Mg ha^{-1} yields in the fall and spring, respectively (Tollenaar et al., 1992). In the same region, interseeding CCs into sweet and seed corn at the V4 – V6 stages produced a maximum of 1.1 Mg ha^{-1} total dry biomass (Belfry and Van Eerd, 2016) while Belfry et al. (2017) reported up to 2.8 and 3.1 Mg ha^{-1} winter CC biomass yields in the fall and spring, respectively. In southern Manitoba, relay- and double-cropped RC, alfalfa, black medic and chickling peas in winter wheat and fall rye systems produced CC biomass at freeze-up which ranged between 0.63 and 1.2 Mg ha^{-}

^{1} (Martens et al., 2001). Li et al. (2015) found that fall rye CC from 52 observations in Alberta had a seasonal mean C input of 0.4 ± 0.4 Mg ha^{-1}. The variability in establishment shows how diverse microclimates between and within provinces, seasonal (temporal) variations, as well as underlying management practices can determine the extent to which CC biomass can accumulate. Although CC biomass accumulation may not always translate to significant impacts on yield or direct economic gains, it is critical for other ESs, particularly SOM buildup and soil health improvement in intensively managed conventional cropping systems.

2.6.2. Crop Yield Responses to Cover Crops

Yield increases are direct on-farm benefits that can assuage the hesitation of farmers who are concerned about potential declines in farm profitability following CC adoption. Some studies in Canada have shown significant positive cash crop yield responses to CCs (Table 2.2). For example, increases in grain corn yields were observed following RC (Coombs et al., 2017; Vyn et al., 1999), alfalfa (Coombs et al., 2017), and winter cereal rye (Ball-Coelho and Roy, 1997) cover crop incorporation in Ontario. Shrestha et al. (2002) also reported higher soybean yields in plots with CCs compared to those without. In Quebec, red and white clover increased canola yield by up to 42 % when no additional N was added (Vaillancourt et al., 2017). Similarly, including oat and timothy grass as CCs in a dry bean, potato, spring wheat, and sugar beet rotation in Alberta increased sugar beet yield by up to 11% (Larney et al., 2016). In Nova Scotia, the combination of CCs and organic amendments (municipal food waste, wood ash, and mussel sediments) produced three times more grapes than the no CC control (Messiga et al., 2016). The identification of positive CC responses is key to the promotion of producer interest in CC adoption.

In some cases, crop yields do not significantly increase or decrease in response to CCs. Studies in Ontario dating back over 20 years ago show that CCs often do not have significant impacts on crop yields. For instance, Bruulsema and Christie (1987) tested 20 cultivars of alfalfa and 10 cultivars of RC as green manures for silage corn production in Ontario, and they observed corn yields equivalent to those achieved with 90 - 125 kg ha^{-1} N. Likewise, Ball-Coelho and Roy (1997) found that broadcasted cereal rye did not reduce, but maintained corn yield in a loamy sand. Results from a field study in Ridgetown, ON, which concurrently assessed crop yields and weed suppression effects of CCs, showed reductions in weed biomass density and richness, with no significant effect on sweet corn yield (O'Reilly et al., 2011). Moreover, the authors reported higher profit margins from CCs of \$600 - \$1350 ha^{-1} compared to no-CC (O'Reilly et al., 2012; O'Reilly et al., 2011).

In Quebec, interseeded CCs did not increase or decrease corn yield (Abdin et al., 1998). More recently, Belfry et al. (2017) reported no significant negative effects of CCs on marketable tomato yields. In fact, oat and oilseed radish showed potential to increase yield, with profit margins of \$960 and \$1320 ha^{-1}, respectively. Maintenance of yields has also been reported in other systems involving the combination of CCs and manure in MB (Thilakarathna et al., 2015), apples in NS (Sharifi et al., 2016), and when transitioning to organic grain corn (Yang et al., 2019). The maintenance of crop yields after CC inclusion may be an important driver of CC adoption as we see climate change impacts on crop yield stability, particularly when farmers combine this objective with obtaining other CC benefits, such as weed suppression or SOM buildup.

Table 2.2. Studies evaluating cover crops in Canadian field cropping systems for the period 1981 – 2021.

Location	Study Type	Soil Type	Year	Cover crop	Cash Crop	Response Variables	Reference
Vauxhall, AB	Field	Aridic Haplocryoll Loam	2000 - 2011	Oat and winter rye	Potato, spring wheat, dry bean, and sugar beet	Weed suppression	Blackshaw et al., 2015
Vauxhall, AB	Field	Sandy loam	2000 - 2011	Fall rye	Potato, spring wheat, dry bean, and sugar beet	Residue cover, erosion control, crop vigor and yield	Larney et al., 2017; Larney et al., 2016
Leithbridge, AB	Field	Typic Haplustoll loam	2003 - 2005	Barley, winter rye, oat, spring barley, spring rye	Dry bean	CC residue, weed density,	Blackshaw, 2008
Vauxhall, AB	Field	Sandy loam	2000 - 2011	Oat and fall rye	Potato, dry bean, and sugar beet	Soil quality (POM, SOM, aggregate stability)	Li et al., 2015
Leithbridge, AB	Field	Clay loam and loam	2003 - 2005	Fall rye, Pika winter triticale, barley, oat	Potato, spring wheat and dry bean	Ground cover, weed suppression, wheat yield	Moyer and Blackshaw, 2009
Leithbridge, AB	Field	Clay loam	2013 - 2014	Fall rye, oilseed radish	Spring wheat	CC biomass, SMN, C accumulation and N uptake, and wheat yield	Thomas et al., 2017
Leithbridge, AB	Field	Clay loam	2013 - 2015	Fall rye, oilseed radish	Spring wheat	N_2O fluxes, WEOC and SMN	Thomas et al., 2017
Fairview, AB	Field	?	2015 - 2016	Variety (cocktails)	Haymaker oat	Forage dry matter yield and nutritional value	Omokanye, 2017
Fraser River Delta, BC	Field and Laboratory	Silty clay loam	1995	Spring barley, fall cereal rye, annual ryegrass	Potato	Aggregate stability, soil organic C, total and dilute acid extractable polysaccharides	Liu et al., 2005

Table 2.2 continued… Studies evaluating cover crops in Canadian field cropping systems for the period 1981 – 2021.

Location	Study Type	Soil Type	Year	Cover crop	Cash Crop	Analysis†	Reference
Fraser River Delta, BC	Field	Silty clay loam	?	Spring barley, fall cereal rye, annual ryegrass	None	Aggregate stability	Hermawan and Bomke, 1997
Winnipeg and Carman, MB	Field	clay and fine sandy loam	1997 - 1999	Red clover, alfalfa, black lentil, and chickling vetch	Oats, winter wheat and fall cereal rye	N fertilizer replacement value	Martens et al., 2001
Winnipeg, MB and Indian Head, SK	Field and growth chamber	Silty clay and heavy clay	2000 - 2006	Black medic	Flax	AMF colonization, flax biomass and N and P uptake	Turmel et al., 2011
Carman, MB	Field	Fine sandy loam	2010 - 2012	Fall rye, oats, barley	Organic dry beans	CC biomass, SMN, dry bean development	Evans et al., 2016
Harrington, PEI	Field	Fine sandy loam	1991 - 1993	Red clover, spring barley	Potato	SMN, potato petiole nitrate-N, tuber yield, nematodes	Sanderson et al., 1999
Macdonald and L'Assomption, QC	Field	Loamy clay and silt loam	1993 - 1994	Fall rye; hairy vetch; annual ryegrass; red, white, subterranean, strawberry, Persian, crimson, and berseem clover; yellow sweet clover; black medic; and alfalfa	Grain corn	Corn yield and yield components	Abdin et al., 1998
Normandin and Saint-Augustine-de-Desmaures, QC	Field	Silty clay and sandy clay loam	2013 - 2015	Red clover, white clover	Canola	CC N content, canola yield and N uptake	Vaillancourt et al., 2017
Nipawin and Melfort, SK	Field	Silty clay/clay	2000 - 2002	Oats, barley	Bromegrass, alfalfa	Forage dry matter yield and nutritional value	Malhi and Foster, 2011
Indian Head, SK	Field	Black Chernozem	2003 - 2011	Black medic	Flax, oats, winter wheat,	Microbial diversity and community structure	Lupwayi et al., 2018

Table 2.2 continued… Studies evaluating cover crops in Canadian field cropping systems for the period 1981 – 2021.

Location	Study Type	Soil Type	Year	Cover crop	Cash Crop	Analysis†	Reference
ON	Lab	No soil	2015	Cereal rye, oilseed radish, red clover, oat, hairy vetch	Winter wheat	Extractable P and N	Cober et al., 2018
Ridgetown, ON	Growth chamber	Commercial potting soil	2016	Red clover	Not applicable	Seedling establishment	Loucks et al., 2018
Elora, ON	Field	Grey-Brown Luvisolic loam	?	Alfalfa and red clover	Grain corn	Crop yield and tissue N content	Bruulsema and Christie, 1987
Elora and Woodstock, ON	Field		1981 - 1984	Cereal rye	Grain corn	Rye planting and termination timing vs corn yield	Tollenaar et al., 1992
Elora and Woodslee, ON	Modeling	Silt loam and Brookston clay loam	1959 - 2015	Red clover	Grain corn, oats, alfalfa, soybean, winter wheat	Corn yield, SOC	Jarecki et al., 2018
Elora and Woodstock, ON	Field	Maryhill loam soil	1982 - 1984	Winter rye	Grain corn	CC and corn yield (effect of tillage)	Raimbault et al., 1990; Raimbult et al., 1991
Elora, ON	Field	loam	1982 - 1984	Cereal rye, winter wheat	Grain corn	Corn yield	Tollenaar et al., 1993
Dehli, ON	Field	Loamy sand	1988 - 1996	Cereal rye, corn	Winter wheat, soybean, white bean, kidney bean	Weed density, crop yield	Shrestha et al., 2002
South-central ON	Field	Typic Hapludalf	1989 - 1995	Annual ryegrass, red clover, oilseed radish,	Winter wheat, barley, and grain corn	Spring SMN, and N availability to corn	Vyn et al., 1999

Table 2.2 continued… Studies evaluating cover crops in Canadian field cropping systems for the period 1981 – 2021.

Location	**Study Type**	**Soil Type**	**Year**	**Cover crop**	**Cash Crop**	**Analysis†**	**Reference**
Dehli Farm, ON	Field	Fox loamy sand	1993 - 1995	Cereal rye	Grain corn	Corn grain yield, corn N uptake, rye root and shoot biomass, SMN (PSNT and N leaching)	Ball-Coelho and Roy, 1997
South-central ON	Field	Typic Hapludalf	1992 - 1995	Cereal rye, oilseed radish, red clover, oat	Winter wheat and grain corn	SMN and corn yield	Vyn et al., 2000
Harrow, ON	Field	Brookston clay loam	1999 - 2003	Winter wheat	Grain corn, soybean	Surface runoff and tile drainage flow volumes and P content	Zhang et al., 2017
Elora and St. Mary's, ON	Field	Loams	2003 - 2004	Red clover, oilseed radish, oat, perennial ryegrass,	Grain corn	Crop yield and manure-N uptake	Thilakarathna et al., 2015
Bothwell and Ridgetown, ON	Field	Brady loamy sand and Brookston sandy loam	2006 - 2008	Oat, cereal rye, oilseed radish	Sweet corn	Weed suppression; Mineral N content, crop yield, and profit margins	O'Reilly et al., 2011; O'Reilly et al., 2012
Ridgetown, ON	Laboratory	Sandy loam	2007 - 2010	Oat, cereal rye, oilseed radish	Peas, sweet corn, spring wheat, tomatoes, field corn, squash	C mineralization	Ouellette et al., 2016
Ridgetown, ON	Field	Sandy loam	2007 - 2016	Oat, cereal rye, oilseed radish	Peas, sweet corn, spring wheat, tomatoes, grain corn, squash, soybeans	Soil health (labile SOC and SON; soil health tests), C sequestration, profit margins	(Chahal and Van Eerd, 2018a; Chahal and Van Eerd, 2019; Chahal and Van Eerd, 2020; Chahal and Van Eerd, 2021; Chahal et al., 2020a; Chahal et al., 2020b)

Table 2.2 continued… Studies evaluating cover crops in Canadian field cropping systems for the period 1981 – 2021.

Location	**Study Type**	**Soil Type**	**Year**	**Cover crop**	**Cash Crop**	**Analysis†**	**Reference**
Ridgetown, ON	Field	Sandy loam	2010 - 2011	Oat, cereal rye, oilseed radish	Peas, sweet corn, spring wheat, tomatoes	CC biomass and C/N ratio, SMN, tomato yield and quality, pest pressure, economic analysis	Belfry et al., 2017
Ridgetown, ON	Field	Sandy loam	2011 - 2014	Oat, winter cereal rye, oilseed radish, forage pea, hairy vetch	Field bean and sweet corn	Crop yield and N dynamics	Van Eerd, 2018
Ridgetown, ON	Field	Loam and sandy loam	2012 - 2014	Crimson clover, red clover, alfalfa	Grain corn and oats	SMN, crop biomass and grain yield, plant tissue C and N content,	Coombs et al., 2017
Ilderton and Londesborough, ON	Field	Silt loam and clay loam	2014 - 2015	Oat and red clover	Winter wheat	Water-extractable P in residues and dissolved reactive P or total P in runoff	Lozier et al., 2017
Harrow, ON	Field	Brookston clay loam	2014 - 2017	Crimson clover, hairy vetch, and red clover	Grain corn, soybean, winter wheat	N and C accumulation in the legume above-ground biomass, residual SMN, grain yields	Yang et al., 2019
London, ON	Field Incubation (mesocosms)	Silt loam	2015 - 2016	Red clover	Arugula	Microbial biomass N, SMN	Zhou et al., 2017
Mull, Blenheim, Harrow, and Morpeth; ON	Field	Sandy loam - loam	2015 - 2017	Oilseed radish, annual ryegrass, oat, crimson clover, cereal rye, pea, triticale, and pea	Grain corn	Suppression of Canada fleabane weed	Cholette et al., 2018
Harrow, ON	Field	Brookston clay loam	2001 - 2018	Red clover	Grain corn, soybean, winter wheat	Soil health (rotation effects)	Agomoh et al., 2020

Regrettably, yield losses following CC inclusion have also been reported in Canada. In Ontario, annual ryegrass was reported to reduce corn yields (Vyn et al., 1999), while winter rye planted after silage corn harvest in a loam soil delayed corn development and led to 11 - 17% reductions in corn biomass yield (Raimbault et al., 1990). Tollenaar et al. (1992) and Tollenaar et al. (1993) reported that crimson clover presented significant competition to grain corn and rye residues caused delays in corn planting date which led to significant reductions in corn yield in Elora and Woodstock, ON. In SK, Malhi and Foster (2011) found that oats and barley reduced forage yield and crude protein, while in MB and SK, black medic CC reduced flax biomass (Turmel et al., 2011).

Low residue decomposition rates of CC residues have been noted to reduce crop yields, as was reported for fall rye which reduced spring wheat grain yields up to 57% (Thomas et al., 2017). In some cases, CCs such as fall rye (Evans et al., 2016) or crimson clover (Abdin et al., 1998) can be overly competitive with the cash crop, leading to reductions in yields. CCs which reduce yields would not be good candidates for systems where the CC is interseeded into a cash crop, unless there are other benefits which can offset the yield loss. For instance, if CCs are used for grazing or harvested as forage, producers may have a direct benefit which could justify continued inclusion of said CC. Research that showcases negative, positive, or neutral impacts on productivity are all important as they can inform producers and researchers who are interested in CCs but are lacking adequate knowledge of viable options.

2.6.3. Weed Suppression, Erosion Control and Other Benefits

Cover crops can indirectly impact yields through their effects on weed biomass, density, and species richness. Only a handful of studies have evaluated weed responses to

CCs in Canadian cropping systems. In ON, CCs reduced weeds and erosion in plots with soybeans, white beans, and kidney beans than in plots with cereals (Shrestha et al., 2002). In a field study involving fall rye, oilseed radish and oat CCs in Ridgetown, ON, O'Reilly et al. (2011) reported reductions in weed biomass density and richness with no significant effects on sweet corn yield. Conversely, in Southern Alberta, Moyer and Blackshaw (2009) reported that CCs grown after potatoes and dry beans produced adequate (>30%) ground cover but did not reduce weed density, and reduced the yield of the succeeding spring wheat crop. Evans et al. (2016) concluded that frost-killed CCs did not provide sufficient weed suppression in organic dry beans in Manitoba. Blackshaw et al. (2015) found no clear effects of oat and winter rye CCs on weed suppression, but the inclusion of CCs significantly reduced erosion in a potato - spring wheat - dry bean - sugar beet rotation. In Alberta, the inclusion of fall rye CC after potato and dry bean led to 30% residue cover, which was sufficient to significantly reduce wind erosion (Larney et al., 2017). Overall, the effectiveness of CC in suppressing weeds or controlling erosion is as variable as yield, and current evidence suggests a need for continued research to identify effective CC niches for different cropping systems and regions.

2.6.4. Changes in Soil and Crop Nitrogen Status Following Cover Crops

The potential for some leguminous CC species to contribute N to succeeding cash crops, the nutrient scavenging potential of some cereals, or the additional C and N inputs obtained from residue return from fast growing species are some of the most sought-after CC benefits. As all benefits vary by CC species and their interaction with climatic and management factors, region-specific research can help farmers and policymakers identify potential CC niches that match their soil improvement objectives without compromising yield. Soil mineral N and crop N content responses have widely been evaluated in most of the published Canadian studies (Table 2.2), and the findings are also as varied as found with yield or establishment. In southern Ontario, Belfry et al. (2017) reported that oilseed radish increased SMN during cover crop growth and during the succeeding tomato growing season, but they did not observe the same increases with winter rye, oat, or an oilseed radish/oat mixture. In their comparison of multiple varieties of alfalfa and RC, Bruulsema and Christie (1987) found that alfalfa residue-N was more readily mineralized than that of RC, and alfalfa increased corn N content following residue incorporation. Thomas et al. (2017) established that wheat yield following fall rye and oilseed radish was limited more by spring N supply than by CC biomass in a 3-yr study. Some longer-term studies show clearer CC effects, for instance, in a 6-yr trial, Vyn et al. (1999) observed greater potential for increased N supply to succeeding corn following RC compared to annual ryegrass. Their results showed that following RC, soil NO_3-N levels were 2.8 times higher and aboveground corn biomass N was 40.4 kg ha^{-1} greater than without a CC. Current results indicate potential for CCs to contribute significantly to soil N fertility or crop N uptake; however, more long-term studies are needed. It appears the soil fertility benefits of CCs are more likely to come from the buildup of SOM, which is usually a medium- to long-term benefit.

2.6.5. Cover Crop Effects on Soil Health

In the past decade more and more studies are devoted to the area of soil health. A team led by Dr. Laura Van Eerd at the University of Guelph has assessed CCs in a trial established in 2007 at Ridgetown, ON (Chahal and Van Eerd, 2018a; Chahal and Van Eerd, 2019; Chahal and Van Eerd, 2020; Chahal and Van Eerd, 2021; Chahal et al., 2020b). From a medium-term (6-yr) CC trial at Ridgetown, Chahal and Van Eerd (2019) evaluated the comprehensive assessment of soil health (CASH) test relative to the Haney soil health test. They reported superior performance of the CASH test developed in ON and produced a minimum dataset with only five indicators, pH, organic matter (OM), Solvita labile amino N, Solvita CO_2-burst, and water-extractable organic C. From their 8-yr trials, CCs significantly increased soil organic C content by 10 - 20 Mg C ha^{-1} (Chahal et al., 2020b). In London, ON, Zhou et al. (2017) set up field mesocosms and reported increases in soil microbial biomass N and N leaching potential in the early spring following RC incorporation. There is also existing evidence that even in the long-term, CC inclusion may not significantly increase SOC or particulate SOM carbon (POM-C) and -N, particularly if C inputs from CC are not substantial relative to the inputs from cash crops or organic amendments (Li et al., 2015). Similarly, Agomoh et al. (2020) found that the effects of cover crops on soil health attributes, such as POM-C, PMN, and active C/permanganate-oxidizable C (POXC), were insignificant when assessed together with rotation effects in a 17-yr trial. These findings suggest the need for system-appropriate choices if CC benefits are to be realized.

2.7. Conclusion

Intensively managed agricultural systems in Canada are highly productive and profitable, and yet pose long-term risks to the environmental sustainability of the agricultural, and surrounding landscapes. There are trends towards reduced crop biodiversity and associated decreased soil health on farms due to market demands focusing on a small number of profitable crops resulting in simplified rotations with low residue inputs. Cultural management practices such as increasing rotation complexity by intercropping and cover cropping can be used to provision ES from cash crop systems. Cover crops have great potential to offer many ESs to agricultural systems, and to also have economic benefits when managed properly. One of the many hindrances to CC adoption is the lack of knowledge on region-specific CC niches as well as benefits for specific cropping systems.

The recognition of the negative trends in environmental indicators associated with cash crop systems, e.g., loss of SOC on Ontario soils (McRae et al., 2000; Smith, 2015), and the need for research to support farmer adoption of CCs (Mervin and McLarty, 2017) are the main driving forces for current renewed interest by producers, scientists, government institutions, and other interest groups in prioritizing CC research. Therefore, scientific research has an opportunity to support the efforts of producers, policymakers, and other stakeholders by providing evidence for potential CC efficacy in the local context (for specific crops or regions). While most of the research to date has focused on the feasibility of establishing CCs between cash crop cycles, and on the impacts on yield and soil mineral N and P dynamics, there remains a wide research gap on the implications of CC inclusion for soil health in Canada. The assessment of CC benefits and temporal and spatial niches in the Canadian agricultural landscape could help incentivize the adoption of CCs, particularly where yield benefits are lacking, inconclusive or contradictory.

3. INTERSEEDED COVER CROPS IN GRAIN CORN: IMPLICATIONS FOR YIELD, NITROGEN UPTAKE AND RESIDUAL SOIL NITROGEN

3.1. Introduction

Grain corn (*Zea mays*) is Canada's third most economically valuable crop, after canola (*Brassica napus*) and wheat (*Triticum* aestivum) (Harnel and Dorff, 2016). Approximately 60% of the 1.3 million hectares of Canada's arable land that is used for grain corn production is in Ontario (Statistics Canada, 2016). More than 70% of grain corn varieties grown in Ontario are genetically modified (Roundup Ready®), facilitating the application of glyphosate-based herbicides which minimize competition from weeds and maintain high yields. Corn production usually involves moldboard plowing and disking, followed by planting in 0.76 m-wide rows in early spring (May) (OMAFRA, 2017). Herbicides often lead to minimal soil cover between the wide corn rows, leaving soil exposed to erosion (OMAFRA, 2017), while tillage enhances SOM decomposition (Shi et al., 2012). One of the main challenges for conventional corn production systems in Ontario is the continued decline of soil health due to intensive tillage and reduced SOM contents (Deen and Kataki, 2003; McRae et al., 2000; Shi et al., 2012).

Strategies are continually sought to support and promote the maintenance of soil health on Ontario farms (Mervin and McLarty, 2017). These include diversification of rotations, reduced, minimum, and no-till management, and integrated weed and pest management. Presently, over 95% of Canada's grain and silage corn are grown in rotation with legumes (mostly no-till soybean), cereals (commonly winter wheat) and hay (Harnel and Dorff, 2016). The most popular rotational systems for grain corn in Ontario are corn-soybean and corn-soybean-wheat (Congreves et al., 2014). However, there is a trend towards simpler and shorter rotations (corn-soybean) (Gaudin et al., 2013; Gaudin et al., 2015), which threatens the diversity and resilience (Ponisio and Ehrlich, 2016) of corn systems in Ontario. However, as producers opt for simpler rotations, it creates an opportunity for inclusion of CCs in corn-based systems.

Traditionally, CCs are planted in what would otherwise be fallow periods, e.g., in the summer following a winter cereal such as wheat, as this allows ample time for CC establishment between cereal harvest and freeze-up. However, with shorter corn-soybean rotations that exclude winter cereals, there is limited time to introduce CCs after cash crop harvest. If CCs are to be adopted in Ontario corn systems, then the window for successful establishment is restricted to the short period between grain harvest and the seeding of the subsequent crop in the following spring. Interseeding CCs into standing corn earlier in the season could ensure more robust establishment before the start of the frost period. Several trials in the US have shown success with newer technologies such as the Interseeder™ Drill (Bich et al., 2014; Roth et al., 2015). With such technologies, research has shown that the inclusion of CCs in corn through interseeding is mostly successful at the V4 – V6 stage when corn plants are well established and competition with the CCs is less likely to be detrimental to yield (Belfry and Van Eerd, 2016; Bich et al., 2014).

Cover cropping benefits are widely acknowledged and have been extensively studied within different cropping systems and climates (Sarrantonio and Gallandt, 2003; Schipanski et al., 2014). Benefits of cover cropping include: protecting soil from wind and water erosion (Blackshaw, 2008; Wall et al., 1991); suppressing weeds (O’Reilly et al., 2011; Teasdale et al,. 2007); minimizing nutrient losses due to leaching (Tonitto et al., 2006) and runoff (Blanco-Canqui et al., 2015; Constantin et al., 2010); supplying N to crops (Kaye et al., 2019; White et al., 2017); and stimulating soil microbial diversity, promoting beneficial soil microbes, and suppressing pathogens in perennial systems (Vukicevich et al., 2016). The use of CCs as a management strategy to improve N use efficiency (NUE; estimated to be in the range 30 - 50% for most field crops) has also been greatly emphasized (Cassman et al., 2002; Drinkwater and Snapp, 2007; Fageria and Baligar, 2005; Smil, 1999). Cover crops could contribute to the improvement of NUE in conventional agriculture if used as a soil conditioning strategy to build SOM (Gabriel et al., 2016; Mulvaney et al., 2009).

Despite all the known potential benefits from CCs, uptake in Ontario has been slow. This is partially because there is still a lot of uncertainty about the viability of CC inclusion (Mervin and McLarty, 2017). Also, there are challenges with establishing CCs in a region that has short windows of opportunity for CC inclusion between growing seasons. Interseeding CCs into standing corn could address some of the challenges with establishment, but little is known about the extent of competition between corn and interseeded CCs for nutrients, especially N (Baumgart-Getz et al., 2012). Cover crops have the potential to retain surplus nutrients after cash crop harvest, thereby minimizing nutrient loss into the environment. However, the extent of CC establishment, amount of biomass accumulated, and impacts on plant available N varies by species, seeding rate as well as soil type (Coombs et al., 2017). The implications of interseeding CCs under conventional N fertilization regimes for corn yields and N supply, i.e., amount of N taken up by the interseeded CCs, the corresponding effects on corn N uptake, as well as soil N status (residual soil mineral N (RSN; NH_4^+ and NO_3^-) at grain harvest and in early spring, have not been widely studied in southern Ontario and warrant investigation.

This study was set up to evaluate the effects of interseeded annual ryegrass and RC on soil N dynamics in southern Ontario grain corn systems. The hypothesis was that interseeded annual ryegrass and RC would not significantly compete for N with corn when interseeded at the V4 – V6 stage; they would accumulate significant amounts of biomass and residue C and N; and would significantly reduce residual SMN to minimize potential leaching or erosion losses. Van Eerd (2018) emphasizes the need for crop-specific recommendations to enable meaningful CC inclusion in cropping systems, without compromising N supply. To establish such recommendations, or to encourage CC adoption, research such as the current study is needed to quantify crop responses to different cover cropping options.

3.2. Materials and Methods

3.2.1. Study Sites, Experimental Design and Treatment Layout

Field trials were established in early May 2015 and repeated in 2016 at two sites in southwestern ON (Figure 3.1): the University of Guelph Ridgetown Campus, Ridgetown (RDG; 42° 27' 3.6972" N, 81° 53' 22.9488" W) and the University of Guelph Elora Research Station, Elora (ELR; 43° 38' 0.2544" N, 80° 23' 19.2480" W); and one site in central ON: the Trent University Experimental Farm, Peterborough (PTB; 44° 21' 42.0588"N, 78°16'42.6000"W). Soils at the sites were classified as: ELR, well drained grey-brown Podzolic Guelph loam (US Classification: Inceptisol); RDG, well drained and gravelly Orthic Humic Gleysolic Fox loam (US Classification: Humaquept); and PTB, poorly drained and stony Orthic Melanic Brunisolic Otonabee loam (US Classification: Hapludoll). At all sites, new experimental plots were established in each of the two seasons, 2015/16 and 2016/17, i.e., each year was a separate trial, and is hereafter referred to as a "site-year". The site-years are designated as ELR15, PTB15, RDG15, ELR16, PTB16, and RDG16 for Elora-2015, Peterborough-2015, Ridgetown-2015, Elora-2016, Peterborough-2015, and Ridgetown-2016, respectively. Plots were established in fields that had previously been seeded to soybean at ELR15, ELR16, RDG15, and RDG16, and to barley at PTB15 and PTB16. Although six site-years were established, only four site-years (ELR15, PTB15, ELR16, and RDG16) had successful CC establishment to provide adequate data for inclusion in this thesis. Details are also provided in section 3.3.1.

At each site-year, the trials were laid out in a randomized complete block design (RCBD) with four blocks, with the site-years and CC treatments as fixed factors. Each season, grain corn was interseeded with one of three CC treatments: RC (RC), annual ryegrass (AR), or a 1:3.3 mixture of RC and AR (MIX) at the V4 – V6 corn stage (4 – 6 leaves). Plots without interseeded CCs were included as controls (NOCC) for comparison, bringing the total number of plots for each site-year to 16. Prior to treatment establishment, soil was collected from the 0 – 15 cm depth from each of the four blocks at each site. Nine soil cores were collected in a zig-zag pattern using an auger and homogenized to form one composite which was characterized for texture, pH, CEC, EC, and soil organic matter (SOM) (Table 3.1). Total C and N were determined by dry combustion on a Vario MAX cube CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) (Rutherford et al., 2007; Skjemstad and Baldock, 2007). Soil texture was measured by the hydrometer method (Kroetsch and Wang, 2008). Phosphorus was determined by the bicarbonate/ascorbic acid extraction method (Schoenau and O’Halloran, 2008). Exchangeable bases, Ca, K, Mg, Al, and Na were measured using ICP-OES following 1M ammonium acetate extraction at pH 7, and cation exchange capacity (CEC) was calculated from the exchangeable bases (Hendershot et al., 2008a). Soil pH and EC were measured in 1:2 v/v soil/water (Hendershot et al., 2008b).

3.2.2. Agronomic Practices

Each of the 16 plots in each site-year was seeded with corn in 0.76 m rows, targeting a final plant density of ~78,000 plants ha^{-1}. Plot dimensions were 3 m × 8 m at Ridgetown and Elora (4 corn rows) and 4.5 m ×12 m at PTB15 (6 corn rows) (Figure 3.2). Sampling of grain corn, CCs, and soils was conducted within the central 4 m^2 of the three innermost corn rows. Table 3.2 shows the main activity dates, corn varieties and sampling dates for the three sites. Starter-fertilizer, sidedress-N, and herbicides were applied according to regional recommendations by OMAFRA (Table 3.2). Immediately following sidedressing with urea ammonium nitrate, three rows of CCs were seeded between the corn rows using an Interseeder™ drill that was manufactured and marketed for interseeding CCs into corn [(Roth et al., 2015); Interseeder Technologies LLC., Woodward, PA, USA]. The AR and RC treatments were planted at 27 and 9 kg ha^{-1}, respectively, while the MIX treatment was seeded at a 22:7 kg ha^{-1} ratio of AR/RC (Table 3.3). Due to a mechanical error at PTB15, the AR treatment was seeded at 77 kg ha^{-1}. Grain corn yield was determined automatically using combines at ELR15, ELR16 and RDG16, and all residues were left in the field, whereas manual harvesting occurred at PTB15 due to a shortage of harvesting equipment. Consequently, all corn residues were removed from the plots. Overall, CCs were grown for 314 – 325 d (from planting to chemical termination) in the four site-years.

3.2.3. Sampling and Analyses

Soil Sampling

Soil sampling for residual SMN at grain harvest (SMNf) was conducted using soil cores from the 0 - 30 cm depth. For analyses of spring SMN concentration (SMNs), samples were collected from the 0-15 cm depth following CC termination. Nine cores were randomly collected from the central 4 m^2 of the plot area and thoroughly homogenized. Subsamples were placed in polyethylene bags and stored on ice in coolers for transportation to the lab. Samples were refrigerated at 4 °C and analyzed within seven days.

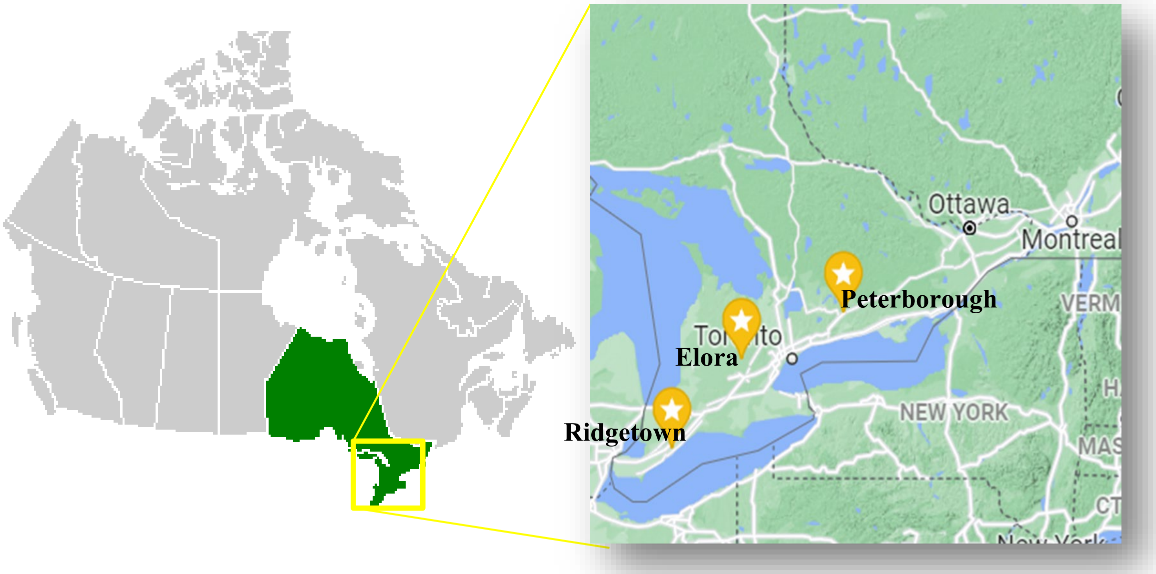


Figure 3.1. Location of study sites at Elora, Ridgetown, and Peterborough in southwestern and central Ontario, Canada. Map sourced from Google Earth ™

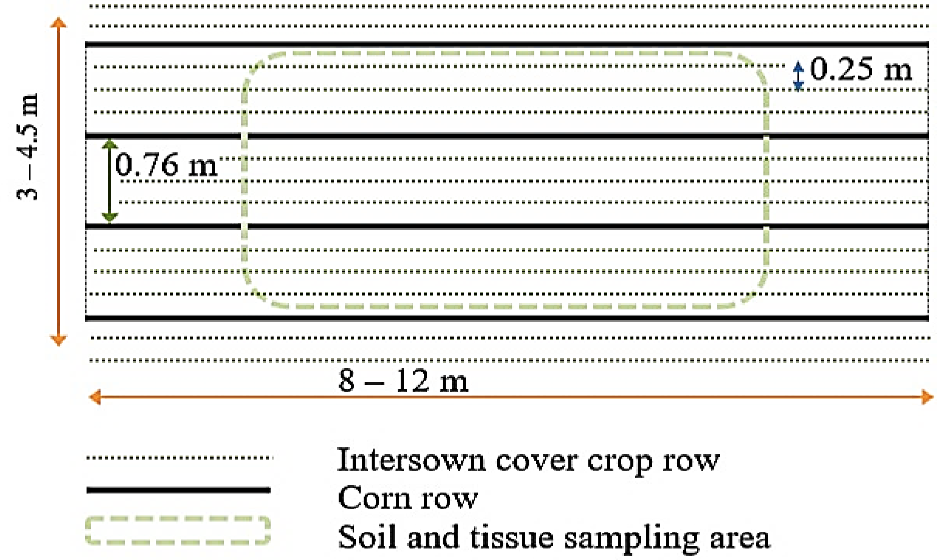


Figure 3.2. Dimensions, sampling area and row spacing of corn and interseeded cover crops.

Grain Corn and Cover Crop Sampling and Analyses

At grain corn maturity (0 – 9 d before harvest), 10 whole corn plants were cut ~10 cm from the soil surface from the middle 4 m of the data rows of each plot and weighed. A 3-plant subsample was randomly selected and weighed for moisture determination. The cobs were separated from the stover, bagged and oven dried at 60 °C for ~72 h. Tissue and grain samples were pulverized using a Retsch Planetary ball mill PM100 (Verder Scientific Inc., Newtown, PA) for total C and N concentration analyses by dry combustion on a vario MAX cube CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Grain yield was converted to a standard grain water content of 15.5% (Cakir, 2004) and N uptake was calculated (Equation 1).

Cover crop and weed aboveground biomass yields were determined three times (Table 3.4): at grain corn harvest (grain harvest), before freeze-up (fall), and prior to termination in the spring with a burndown herbicide (spring). Total CC biomass was the sum of the average of 'grain harvest' and 'fall' biomass, and 'Spring' biomass. An average was used for the total fall biomass due to desiccation and high variability (sparse/non-uniform establishment). At each sampling time, shoots were clipped ~2 cm above the soil surface from randomly selected 0.25 m^2 quadrats. Weeds were also collected within the same quadrat. The plant samples were processed and analyzed as described above for corn grain and stover.

3.2.4. Soil Mineral Nitrogen Determination

Soil mineral N was determined by extraction of 5 g sieved (<2mm) field-moist soil with 50 mL 2 M KCl followed by flow injection analysis (Maynard and Kalra, 2008) using a continuous flow analyzer (Bran Luebbe AA3, Seal Analytical Inc., Mequon, WS).

Nitrogen Uptake

Corn N uptake (NU) was calculated as the sum of stover, and grain uptake calculated as:

$$\mathrm{NU}(\mathrm{kg\ ha^{-1}}) = \sum(\mathrm{Yield} \times \mathrm{N_{conc}}) \qquad [1]$$

where Yield (kg ha^{-1}) is for either stover or grain, and N_{conc} is the corresponding tissue N concentration (kg kg^{-1}).

3.2.5. Statistical Analyses

Yield and soil and tissue analyses data were subjected to two-way ANOVA using the GLIMMIX Procedure of SAS v. 9.4 (SAS Institute, 2016). For each response variable, site-year, CC, and their interaction were modelled as fixed effects, whereas the blocks and interactions of blocks with fixed effects were random effects. Proc UNIVARIATE was simultaneously performed to assess normality of residuals based on the Shapiro-Wilk statistic. For parameters with data that violated ANOVA assumptions (homogeneity of variance or normality of residuals) a natural log transformation was performed. Means were compared using the Tukey-Kramer multiple mean-comparison procedure at the 5% significance level for all pairwise comparisons. Correlation analyses were done using the CORR Procedure at $P < 0.05$. Data were plotted in OriginPro 2020.

Table 3.1. Soil characteristics for the selected sites at the start of the field experiment (n=4).

Parameter†	**2015/16 Season**		**2016/17 Season**	
	Elora	Peterborough	Elora	Ridgetown
pH	7.35 (0.07)†	7.50 (0.01)	7.25 (0.07)	6.70 (0.28)
OM, g kg^{-1}	34.0 (1.41)	36.0 (1.41)	37.0 (1.41)	31.5 (0.71)
Total N, g kg^{-1}	2.09 (0.1)	2.12 (0.24)	1.91 (0.17)	1.58 (0.21)
Total C, g kg^{-1}	24.2 (0.5)	31.9 (1.8)	22.0 (0.68)	20.2 (0.4)
PO_4-P, mg kg^{-1}	16.0 (1.41)	15.0 (1.41)	27.0 (4.3)	20.0 (2.83)
Calcium, mg kg^{-1}	2340 (99)	4345 (304)	2595 (134)	1515 (35.4)
Magnesium, mg kg^{-1}	343 (24.8)	85.0 (4.8)	323 (17.7)	170 (35.4)
Potassium, mg kg^{-1}	66.5 (2.12)	75.0 (0.01)	101 (3.54)	105 (1.41)
Aluminum, mg kg^{-1}	642 (19.8)	483 (11.3)	653 (7.07)	479 (19.8)
CEC, meq 100g^{-1}	14.7 (0.71)	22.7 (1.56)	16.4 (1.13)	10.5 (0.42)
Texture	Silt loam	Silt loam	Silt loam	Sandy Loam
Clay, %	2.98 (0.18)	2.54 (0.03)	2.96 (0.33)	2.35 (0.08)
Silt, %	61.9 (0.02)	53.7 (0.45)	63.0 (0.32)	43.8 (1.65)
Sand, %	35.1 (0.20)	43.6 (0.45)	34.0(0.66)	53.8 (1.56)

† values in parentheses represent the standard deviation of four replicates

Table 3.2: Corn planting and harvesting dates, and fertilizer rates at Elora (ELR), Ridgetown (RDG) and Peterborough (PTB) in the 2015/16 and 2016/17 seasons

Site-year	**Corn Variety**	**Corn Planting Date**	**Grain Harvest Date**	**Starter Fertilizer Rate†**	**Sidedress N Rate**	**Sidedress N Date**	**Herbicide Application Date**	**Herbicide**	**Herbicide Rate**
				kg ha^{-1}					g ha^{-1}
ELR15	DKC 39-97 RIB	May 8	Oct. 22	11.3 - 45 - 34.8	163	June 4	May 23	Glyphosate	362
							June 11	Glyphosate	918
PTB15	Pride A5909 G2 RIB	May 30	Nov. 5	5.8 - 64.1 - 33	126	July 7	June 20	Glyphosate	146
							July 21	MCPA ester 600	480
ELR16	DKC 39-97 RIB	May 10	Oct. 26	7.85 - 31.4 - 31.4	150	June 15	June 3	Glyphosate	1350
RDG16	Pioneer P0216AM	May 6	Oct. 26	13.4 - 54 -27	101	June 13	April 27	Glyphosate	151
							June 15	Glyphosate	151

† N-P-K rates (P as P_2O_5 and K as K_2O)

Table 3.3. Cover crop (CC) seeding, sampling and termination dates, and herbicides applied.

Site-year	**Planting**	**Sampling Dates†**			**Termination**	**Herbicide**	**Herbicide Rate**
		Grain Harvest	**End-of-Fall**	**Spring**			
							g ha^{-1}
ELR15†	June 17	Oct. 14	Nov. 16	Apr. 27 – 28	May 2	Glyphosate	2700
PTB15	July 16	Oct. 27	Nov. 16 – Dec. 4	May 2 – 3	May 25	Glyphosate	432
						Glufosinate-ammonium	640
ELR16	June 20	Oct. 26	Nov. 19	May 3	May 11	Glyphosate	2025
					May 19	Saflufenacil	59
RDG16	June 15	Oct. 24	Nov. 18	Apr. 25	Apr. 25	Glyphosate	2160

† ELR is Elora, PTB is Peterborough, and RDG is Ridgetown; numbers denote year

3.3. Results

3.3.1. Precipitation and Temperature Trends at Study Sites

In the first season of the field trials, seeding operations at PTB started later than at the ELR and RDG sites due to delayed drying of the fields. Additionally, there was a prolonged period of late season drought at Ridgetown which led to desiccation of CCs, hence data from this site was excluded from analyses. For the 2016/17 season, all sites experienced unusually hot and dry conditions (Figure 3.3). The daily temperature and precipitation trends for both seasons are shown in Figure 3.4. In May and June 2016, abnormally high temperatures coupled with below normal rainfall (Figure 3.5) caused a pronounced early-season drought across southern Ontario which led to poor establishment of CCs. Of the three sites, PTB was hit the hardest, and RC did not germinate, therefore data from this site was also excluded from analyses.

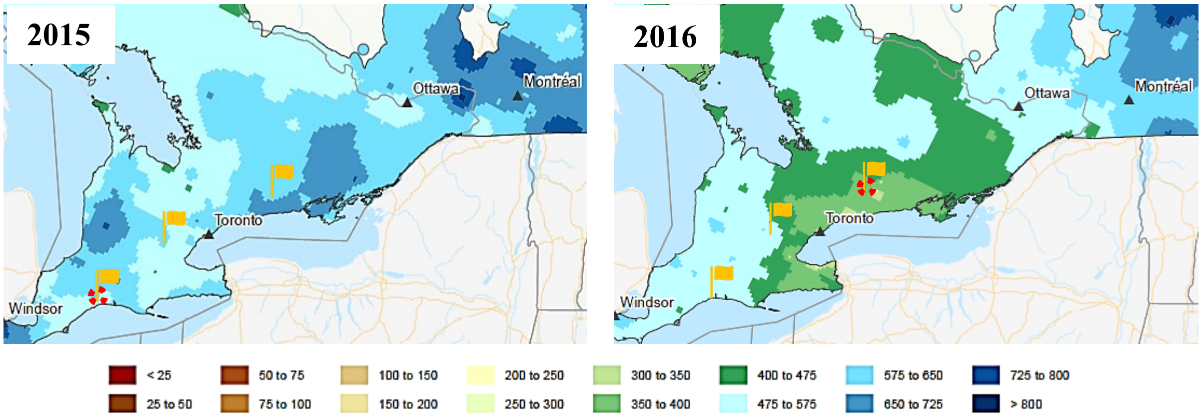


Figure 3.3. Cumulative precipitation (mm) for the 2015 and 2016 growing seasons (Apr. 1 – Oct. 31) at the three study sites in southern Ontario. Map source: (AAFC, 2018) http://www.agr.gc.ca/atlas/agclimate. Due to drought (indicated as ✣), RDG15 and PTB16 had at least one CC treatment fail to establish or maintain biomass.

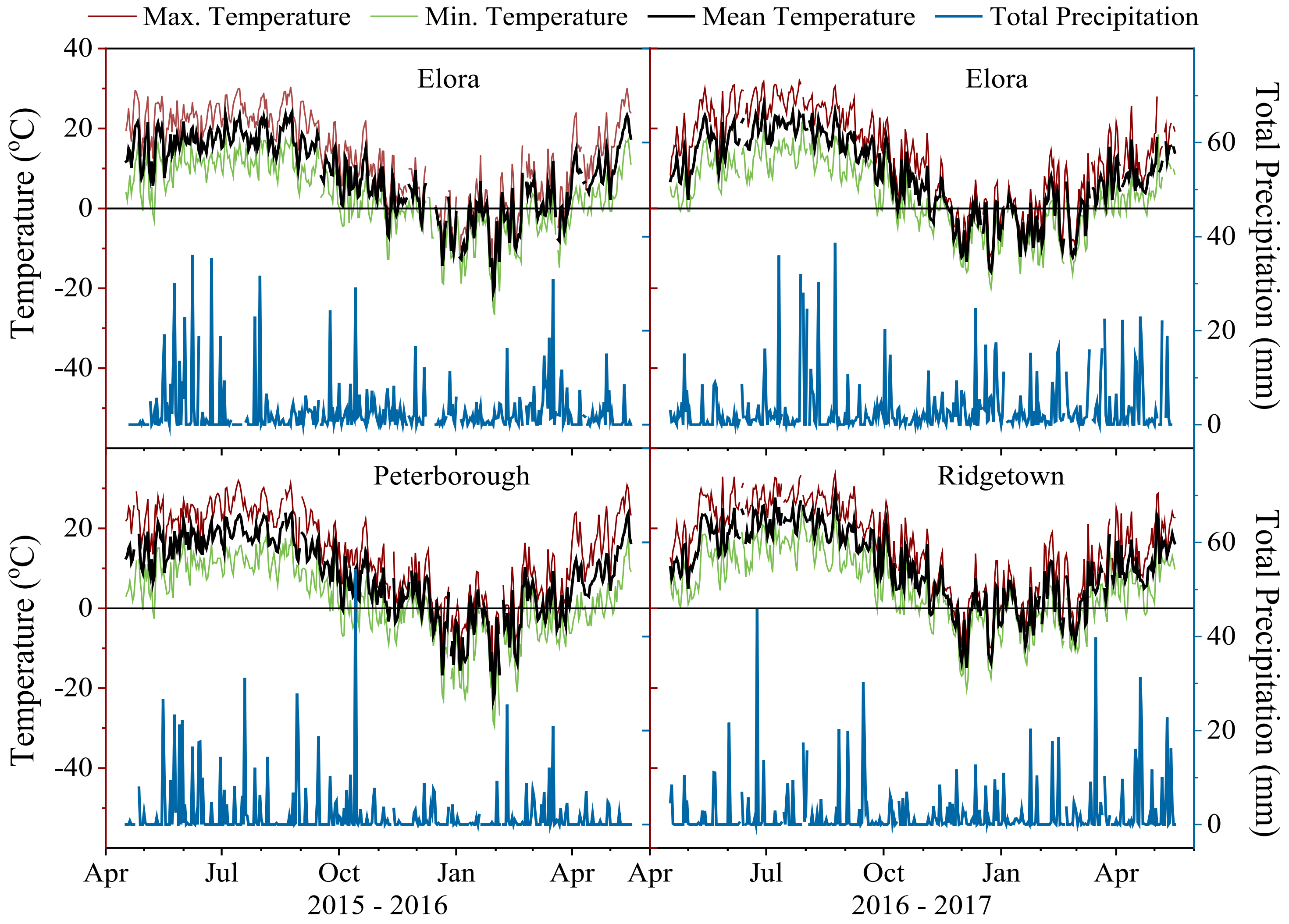


Figure 3.4. Daily precipitation (mm) and temperature (°C) for the 2015 - 2017 growing seasons at Elora, Peterborough, and Ridgetown sites. Historical climate data were obtained from Environment and Climate Change Canada

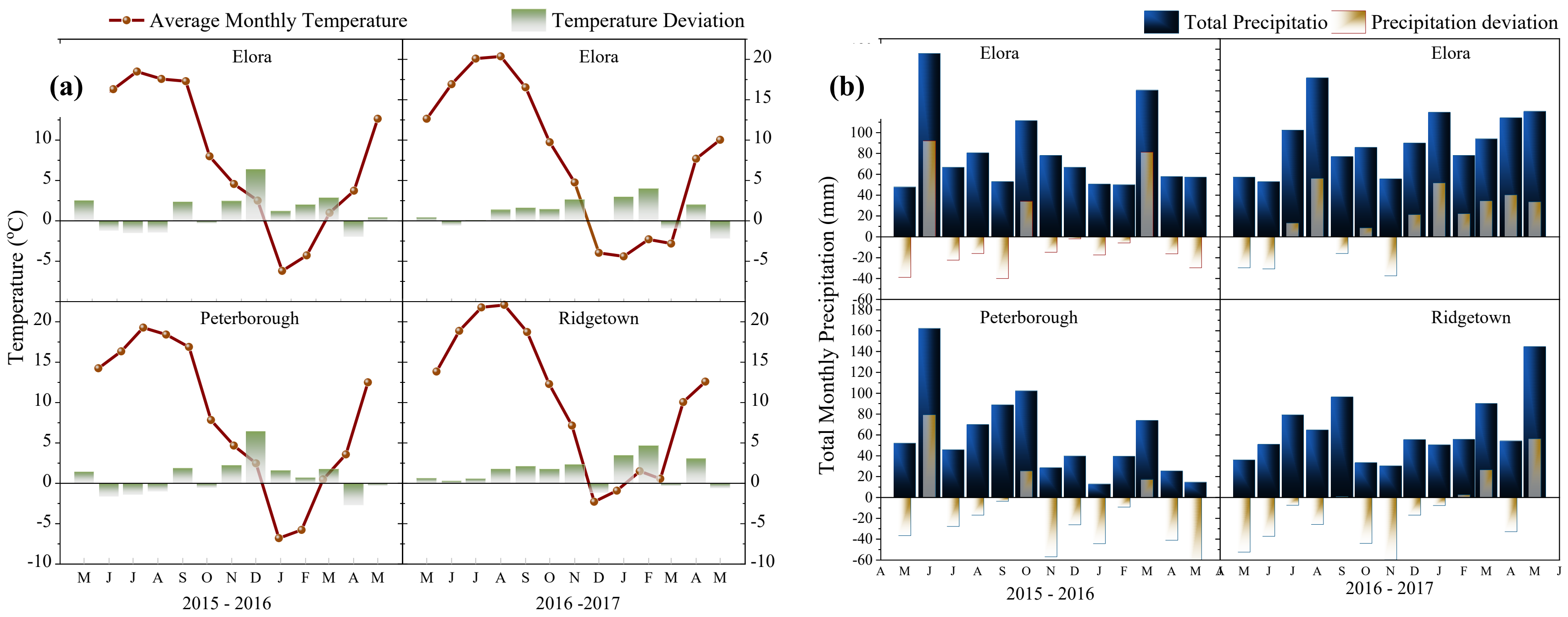


Figure 3.5. Deviations from 20 – 30-yr normals (1981 – 2010) for (a) average monthly temperatures and (b) cumulative precipitation at the four site-years in Southern Ontario. All data were obtained from Environment and Climate Change Canada. Climate normals for Elora were obtained from the Fergus Stand Dam (~12 km from study site) and those for Ridgetown were obtained from New Glasgow (~20 km from study site).

3.3.2. Yield

Corn Grain and Stover

Corn grain yield significantly varied with site-year ($p < 0.0001$; Table 3.4). In 2015, grain yield at ELR15 (11.5 Mg ha^{-1}) was significantly greater than at PTB15 (7.5 Mg ha^{-1}). Conversely, grain yield in 2016 was not significantly different between ELR16 and RDG16 (11.5 and 12.3 Mg ha^{-1}, respectively). Similarly, stover yield was different among site-years, decreasing in the order ELR15 ≥ RDG16 ≥ PTB15 ≥ ELR16. There were no significant effects of CCs on corn grain or stover yield (Table 3.4).

Cover Crop Biomass

Cover crop aboveground biomass yields at all sampling times (grain harvest, fall, and spring) were significantly affected by a site-year × CC interaction ($p < 0.0001$; Table 3.4 3.5). Across the four site-years, CC biomass ranged between 0.45 and 1.6 ± 0.07 Mg ha^{-1} (Figure 3.5).

At ELR15, there were no significant differences in CC biomass between CC treatments, but RC produced significantly greater biomass than AR and MIX at PTB15. For all CCs, there was significantly greater biomass at PTB15 than at ELR15. In the 2016/17 season, there were no significant differences in CC biomass between ELR16 and RDG16 for all CCs. However, RC produced significantly less biomass than AR and MIX at ELR16, but no significant differences in biomass were observed among the CCs at RDG16.

Table 3.4. Corn grain and stover yields, nitrogen uptake, and residual soil mineral nitrogen responses to interseeded cover crops in Southern Ontario.

Effect	**Corn Grain**				**Corn Stover**				**Corn N Uptake**
	Yield†	**N Content**	**C Content**	**C/N Ratio**	**Dry matter yield**	**N Content**	**C Content**	**C/N Ratio**	
	Mg ha^{-1}	g kg^{-1}			Mg ha^{-1}	g kg^{-1}			kg ha^{-1}
Site-Year (SY)									
ELR15	11.5 a‡	10.6 c	413 c	39.2 a	7.62 a	5.55 b	439 a	78.6 a	159 b
PTB15	7.45 b	12.1 b	418 bc	35.2 b	6.81 ab	5.52 b	420 c	77.7 a	118 c
ELR16	12.3 a	13.8 a	433 a	31.6 c	6.29 b	7.63 a	427 b	56.5 b	204 a
RDG16	11.5 a	14.2 a	426 ab	31.1 c	7.66 a	6.97 a	437 a	64.4 b	206 a
Standard Error	*0.38*	*0.45*	*3.47*	*1.12*	*0.26*	*0.22*	*1.18*	*2.55*	*8.08*
Cover Crop (CC)									
Annual ryegrass	10.9	12.1	424	35.7	7.46	6.31	432	70.8	169
Mixture	10.6	12.2	420	35.1	6.84	6.65	430	66.2	168
Red clover	10.6	13.3	422	32.7	6.84	6.61	431	67.5	177
No cover	10.6	12.9	423	33.7	7.24	6.10	430	72.8	173
Standard Error	*0.38*	*0.45*	*3.47*	*1.12*	*0.26*	*0.22*	*1.18*	*2.55*	*8.08*
					p-value				
SY	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CC	0.92	0.10	0.84	0.10	0.18	0.25	0.48	0.22	0.79
SY × CC	0.90	0.32	0.76	0.40	0.11	0.34	0.79	0.64	0.58

† Grain yield was corrected to 15.5% moisture content.

‡ For each main effect, values followed by the same letter are not significantly different according to the Tukey-Kramer multiple mean comparison procedure ($p<0.05$). Main effect mean separation letters are not included for variables with significant site-year × CC interactions; these are plotted in graphs

Table 3.5. Cover crop aboveground biomass yields and carbon and nitrogen contributions, and weed biomass at Elora (ELR), Peterborough (PTB), and Ridgetown (RDG).

Effect	Cover Crops									Weeds			
	Grain harvest†	**End-of-Fall**	**Spring**	**Total§**	**N Content**	**C Content**	**C/N Ratio**	**Total N**	**Total C**	**Grain harvest**	**End-of-Fall**	**Spring**	**Total**
	kg ha^{-1}				g kg^{-1}			g kg^{-1}		kg ha^{-1}			
Site-Year (SY)													
ELR15	189‡	389	587		25.8	302 b	11.8 b	15.3	179	37.9 b	31.3	39.8 b	74.4 c
PTB15	252	566	1204	1613	23.5	354 a	16.1 a	41.3	568	197 a	441	57.2 b	393 a
ELR16	160	254	245	452	24.1	389 a	18.1 a	8.99	179	54.6 b	56.3	136 a	192 b
RDG16	106	117	389	500	26.0	380 a	14.8 ab	12.5	193	38.6 b	21.2	40.7 b	70.6 c
Standard Error	*19.0*	*39.4*	*58.4*	*72.3*	*1.06*	*9.37*	*1.03*	*2.22*	*31.1*	*12.0*	*17.4*	*20.8*	*27.7*
Cover Crop (CC)													
Annual ryegrass	184	297	467	708	20.5	357	18.2 a	13.8	257	59.8 b	113	58.2 ab	145 b
Mixture	176	270	572	795	29.0	341	11.8 b	25.5	287	72.9 b	133	37.0 b	140 b
Red clover	184	277	630	860	25.1	371	15.6 a	19.4	294	67.1 ab	109	59.0 ab	147 b
No cover	-	-	-	-	-	-	-	-	-	128 b	194	120 a	298 a
Standard Error	*15.6*	*35.6*	*54.0*	*66.9*	*0.92*	*7.87*	*0.91*	*2.03*	*28.4*	*12.0*	*17.4*	*20.8*	*27.7*
	p-value												
SY	< 0.001	< 0.001	< 0.001	< 0.001	0.25	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003	< 0.001
CC	0.93	0.79	0.02	0.09	< 0.001	0.06	< 0.001	< 0.001	0.45	0.01	0.02	0.028	0.001
SY × CC	< 0.001	< 0.001	< 0.001	< 0.001	0.001	0.74	0.06	< 0.001	< 0.001	0.10	0.038	0.06	0.17

† Grain harvest sampling was done ≤ 7 d prior to grain corn harvesting

‡ Main effect mean separation letters are not included for variables with significant site-year × CC interactions; these are plotted in graphs. For each main effect, values followed by the same letter are not significantly different according to the Tukey-Kramer multiple mean comparison procedure ($p<0.05$)

§ Totals are the sum of the fall biomass (average of grain harvest and end-of-fall sampling) and spring biomass

Table 3.6. Effects of site-year and cover crop type on soil mineral N and corn and cover crop tissue C and N concentrations.

Effect	**Residual Soil Mineral N**	
	Fall	**Spring**
Site-Year (SY)	mg kg^{-1}	
ELR15	6.70 c†	2.74 d
PTB15	6.93 c	8.26 c
ELR16	32.3 a	15.4 b
RDG16	20.9 b	19.0 a
Standard Error	*1.44*	*0.69*
Cover Crop (CC)		
Annual ryegrass	14.7	10.2
Red clover	16.6	12.1
Mixture	18.9	11.7
No cover	16.7	11.4
Standard Error	*1.44*	*0.69*
	p-value	
SY	< 0.001	< 0.001
CC	0.25	0.16
SY × CC	0.72	0.12

† For each main effect, values in the same column followed by the same letter are not significantly different according to the Tukey-Kramer multiple mean comparison procedure $p<0.05$).

Native Weed Biomass

There was a significant site-year × CC interaction on weed biomass in the fall (p = 0.002; Table 3.5). Conversely in the spring, there were significant site-year (p = 0.003) and CC (p = 0.03) effects. The total weed biomass also showed significant site-year (p < 0.001) and CC (p = 0.001) effects. Averaged for all CCs, total weed biomass was greatest at PTB15, which was two-fold compared to ELR16. Averaged across site-years, CCs significantly reduced total weed biomass by 52% compared to NOCC.

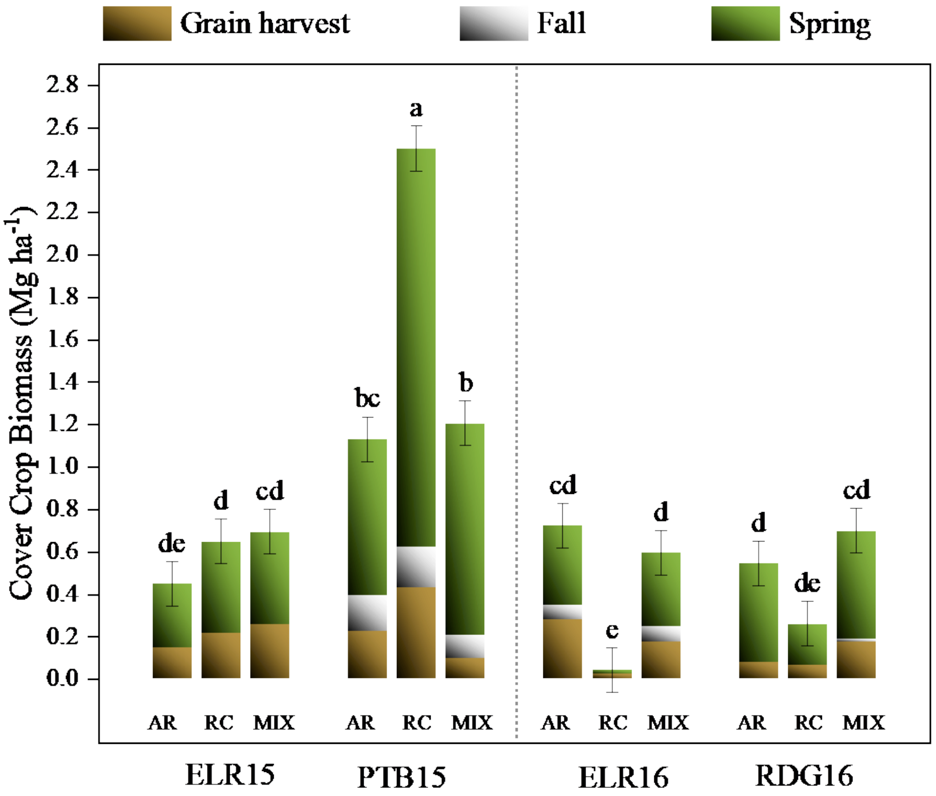


Figure 3.6. Aboveground biomass yields of cover crops harvested before corn harvest (Grain harvest), at the end of the season (Fall), and prior to termination (Spring) (n = 4). Columns with the same letter are not significantly different in total seasonal biomass. Error bars represent the standard error of the mean (P< 0.05).

3.3.3. Carbon and Nitrogen in Corn

Corn Grain

Cover crop type did not significantly affect grain N concentration (range: 12.1 – 13.3 g N kg^{-1}) or C concentration (range: 420 – 424 g C kg^{-1}). However, there was a significant site-year effect on corn grain N and C concentrations (Table 3.4). Grain N was 14% greater at PTB15 than at ELR15, but there was no significant difference between ELR16 and RDG16 (13.8 and 14.2 g N kg^{-1}, respectively). Averaged for all CC treatments, grain N was significantly higher in 2016 than in 2015 (RDG16 = ELR16 > ELR15 > PTB15). There were no significant differences in grain C concentrations between sites in the same season i.e., ELR15 vs PTB15 or ELR16 vs RDG16. However, grain C in PTB15 corn was 3.65% lower than that in ELR16 corn, while RDG16 corn had 4.8 and 3.1% greater grain C than ELR15 and ELR16, respectively. Grain C/N ratios varied significantly by site-year but were not affected by CC (Table 3.4). In the first season, grain C/N ratio at PTB15 was 11% lower than at ELR15. Both sites had significantly higher grain C/N than ELR16 (31.6) and RDG16 (31.1) in 2016.

Corn Stover

Stover N concentration was significantly different between mean site-years but did not differ between CC types (Table 3.4). The 2016/17 sites had significantly greater stover N concentration than the 2015/16 sites. No significant differences were observed between sites within the same season. Stover N concentration ranged from 5.52 to 7.63 g kg^{-1} and varied in the order: ELR 2016 ≥ RDG 2016 > ELR 2015 = PTB15. In the same manner, stover C concentration significantly varied by site-year, but the differences were only between site-years within the same season – i.e., ELR15 > PTB15 and RDG16 > ELR16. Stover C/N ratios were also affected by site-year and followed similar trends as stover N concentration. No differences in stover C/N ratios were observed between PTB15 and ELR15 or between ELR16 and RDG16. Averaged across all CCs, stover produced at PTB15 and ELR15 (77.7 and 78.6 C/N ratios, respectively) had significantly greater C/N ratios than that produced at ELR16 and RDG16 (56.5 and 64.4 C/N ratios, respectively).

Corn Nitrogen Uptake

Corn NU was not affected by CCs but significantly varied by site-year (Table 3.4). In 2015, NU at ELR was 35% greater than at PTB. Both PTB15 and ELR had significantly lower NU than ELR16 and RDG16, which did not differ.

3.3.4. Carbon and Nitrogen in Cover Crops

There was a significant site-year × CC interaction on CC tissue N concentration (CC-N) (Figure 3.7). At ELR, there were no significant differences among the CCs in the 2015/16 season. In the same season at PTB, CC-N varied in the order RC $\geq$ MIX $\geq$ AR. The CC-N in RC (30.7 g kg^{-1}) was 84% greater than AR (16.7 g kg^{-1}), while MIX did not significantly differ from the single-species CC treatments. No differences were observed among the CCs at RDG (22.4 - 28.5 g N kg^{-1}). Overall, there were no significant differences across the site-years for each CC type. Cover crop C concentrations did not differ among AR, RC, and MIX (341 – 371 g kg^{-1}) but varied among site-years (Figure 3.7). On average, CCs at PTB15 contained 17.2% more C than those at ELR15, whereas there was no significant difference between CC-C at ELR16 (389 g kg^{-1}) and RDG16 (380 g kg^{-1}).

Cover crop C/N ratios significantly varied by site-year and CC type (P < 0.01; Table 3.5). Averaged for all CCs, C/N ratios of CCs at ELR15 were 27% lower than those at PTB15, whereas the C/N ratios for CCs at ELR16 were not significantly different from those at RDG16. Across all site-years, RC (11.8) had a significantly lower C/N ratio than both AR (18.2) and MIX (15.6), which were not significantly different from each other.

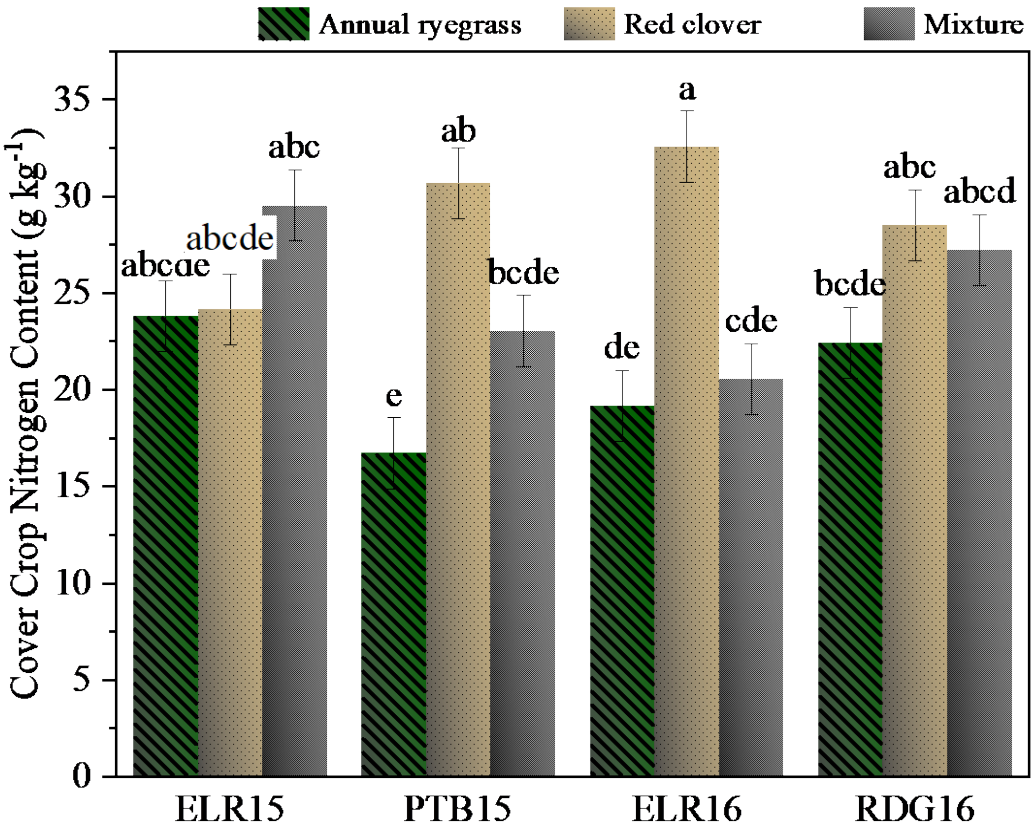


Figure 3.7. Nitrogen concentrations in CCs grown at Elora (ELR), Peterborough (PTB), and Ridgetown (RDG) in the 2015/16 and 2016/17 seasons. Error bars represent standard errors on the mean; columns with the same letters are not significantly different according to the Tukey-Kramer mean separation procedure (P<0.05)

Accumulated Carbon and Nitrogen in Cover Crop Aboveground Biomass

There were significant site-year × CC interactions on total accumulated C and N in aboveground CC biomass ($P < 0.001$; Table 3.5). The total CC-C and -CC-N between site-years varied in the same manner (Figure 3.8). At ELR15, there were no significant differences in accumulated C and N among the CCs. Conversely, significantly less C and N were accumulated from AR (53% C and 76% N) and MIX (48% C and 64% N) than from RC at PTB15. At ELR16, RC accumulated significantly less C than AR or MIX. RC accumulated the greatest amounts of N and C (860 and 77.2 kg ha^{-1}) at PTB15, and the lowest (15.0 and 1.29 kg ha^{-1}) at ELR16.

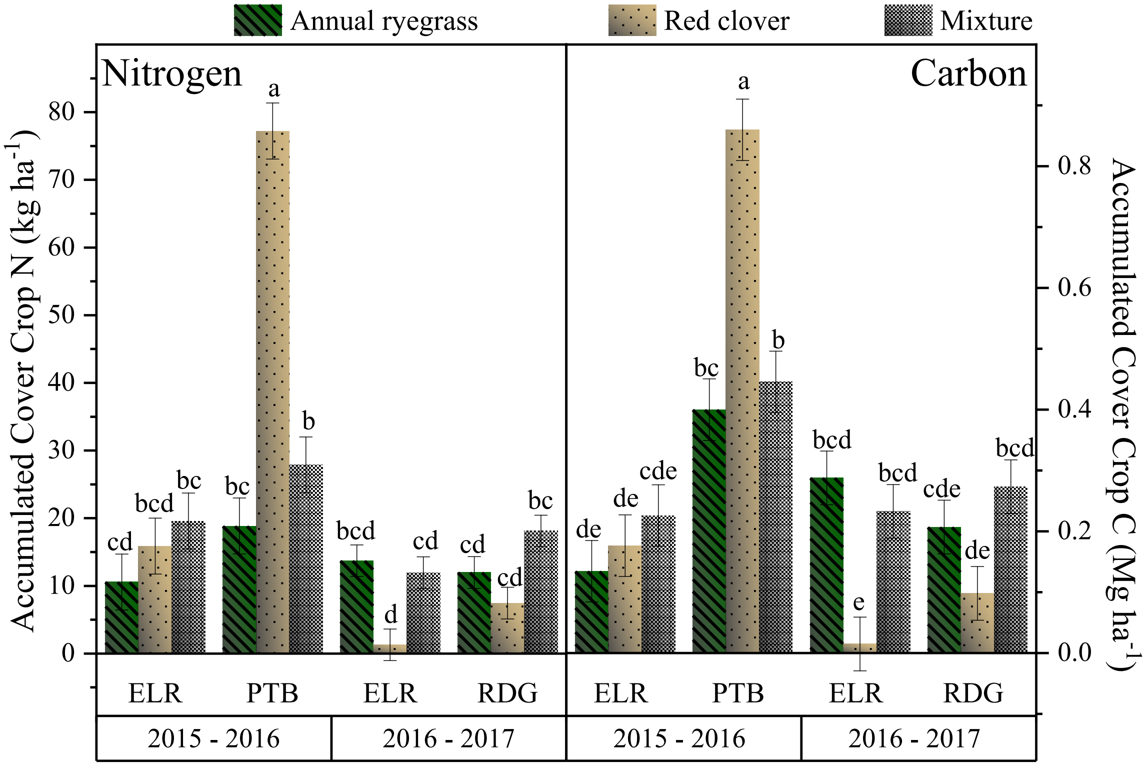


Figure 3.8. Accumulated C and N from annual ryegrass, red clover and their mixture at Elora (ELR), Ridgetown (RDG), and Peterborough (PTB). Error bars represent standard errors on the mean; columns with the same letters are not significantly different according to the Tukey-Kramer mean separation procedure ($P<0.05$).

3.3.5. Soil Mineral Nitrogen

There were significant site-year effects ($P < 0.01$) on SMNf and SMNs (Table 3.5). Overall, both SMNf and SMNs were significantly greater in 2016 than 2015. There were no significant differences due to CCs on either SMNf or SMNs. Averaged across all CCs, SMNf varied in the order: ELR16 > RDG16 > PTB15 ≥ ELR15. Soil mineral N content in the spring was significantly lower at ELR15 and ELR16 than at PTB15 and RDG16, respectively.

3.3.6. Correlation and Regression Analyses

Corn vs Cover Crops

There were significant negative correlations between grain yield, grain C concentration, stover C concentration, and corn N uptake with fall, spring, and total CC biomass ($r = -0.31 – 0.69$; Table 3.6). Stover N concentration had a significant negative relationship with spring and total CC biomass, while stover C/N ratio was positively correlated with CC biomass in the spring. Grain yield, stover C concentration, and corn N uptake had significant negative correlations with the total C and N accumulated over the CC growth cycle. On the other hand, grain N and C concentrations, stover N concentration, and corn N uptake were positively correlated with CC-C concentration.

No corn yield or quality attribute had a significant relationship with CC tissue N concentration. Grain N concentration and C/N respectively had significant positive and negative correlations with CC-C/N, respectively. Stepwise regression showed that CC biomass did not explain significant variation in grain yield but fall weed biomass accounted for 56% of the variation in grain yield. On the other hand, grain C and stover C collectively accounted for 53% of the variation in fall CC biomass (Figure 3.9). The variation in spring CC biomass was strongly related to fall CC biomass and fall weed biomass (Figure 3.10).

Table 3.7. Pearson correlation coeffients for corn grain and stover yield and quality (C and N) with soil mineral N and cover crop attributes (n = 46 - 48)

	Grain				**Stover**				**Corn N Uptake**
	Yield	**N**	**C**	**C/N Ratio**	**Yield**	**N**	**C**	**C/N Ratio**	
Fall CC Biomass	-0.51*†	-0.15	-0.44*	0.12	-0.19	-0.24	-0.56*	0.16	-0.46*
Spring CC Biomass	-0.67*	-0.13	-0.40*	0.07	-0.15	-0.37*	-0.53*	0.30*	-0.57*
Total CC Biomass	-0.66*	-0.14	-0.43*	0.09	-0.17	-0.35*	-0.56*	0.27	-0.56*
Cover Crop C Concentration	-0.03	0.61*	0.36*	-0.63*	-0.36*	0.39*	-0.18	-0.39*	0.31*
Cover Crop N Concentration	0.01	0.00	0.00	-0.02	0.09	0.00	0.11	-0.02	0.02
Cover Crop C/N Ratio	-0.04	0.32*	0.19	-0.31*	-0.26	0.25	-0.20	-0.23	0.13
Total CC-C	-0.56*	-0.05	-0.17	-0.01	-0.09	-0.15	-0.34*	0.15	-0.39*
Total CC-N	-0.51*	-0.08	-0.14	0.03	-0.12	-0.20	-0.31*	0.20	-0.40*
Fall SMN	0.49*	0.58*	0.55*	-0.55*	-0.28	0.69*	-0.03	-0.65*	0.66*
Spring SMN	0.28	0.68*	0.50*	-0.66*	-0.28	0.64*	0.00	-0.58*	0.58*

†Significant Pearson correlation coefficients are indicated by an asterisk (*)

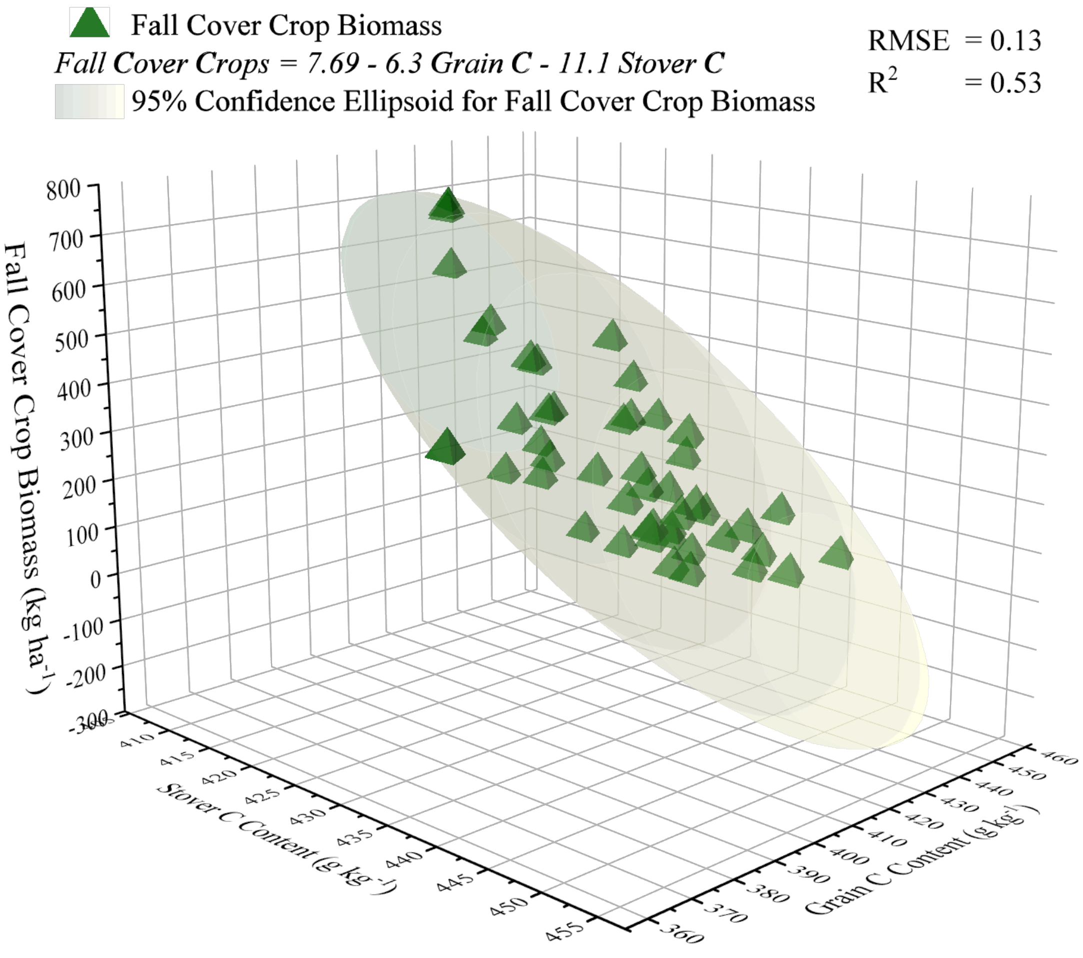


Figure 3.9. Regression analyses of fall cover crop biomass and corn grain and stover C concentrations

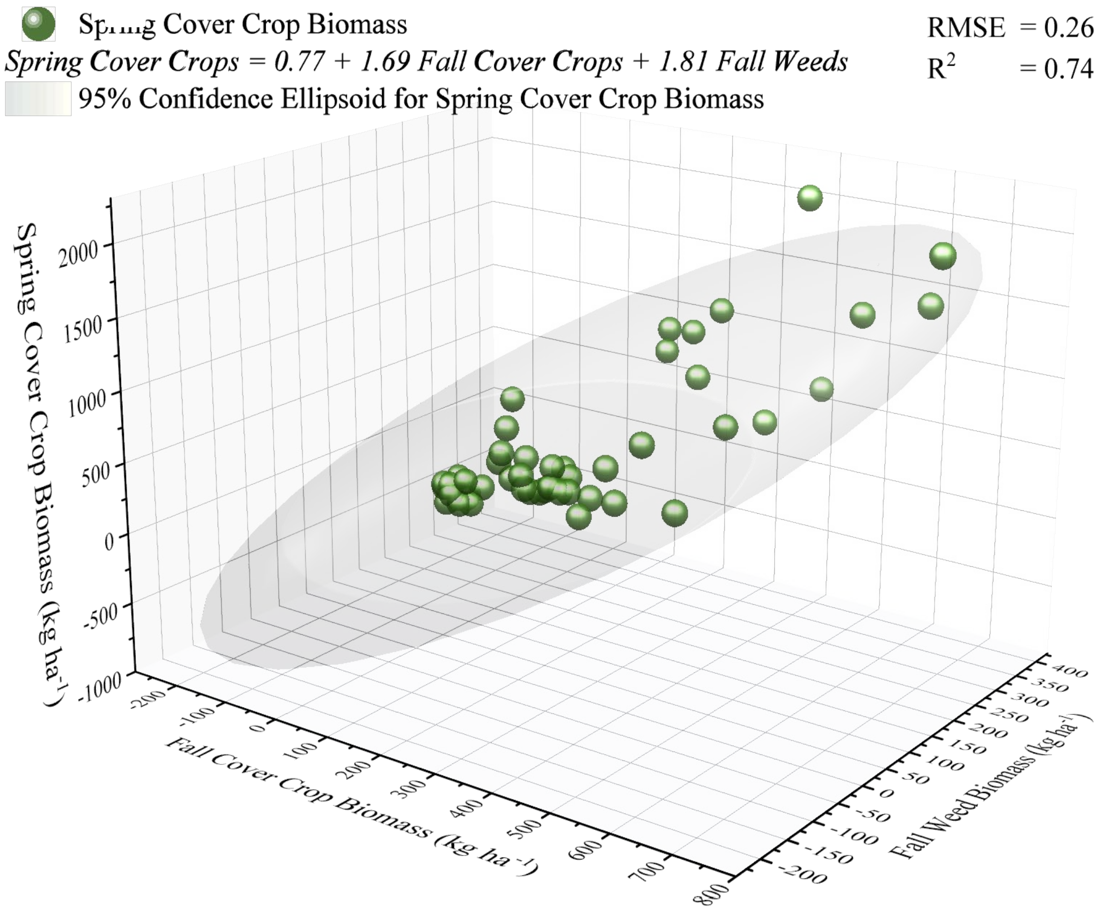


Figure 3.10. Spring cover crop biomass as a function of fall cover crop and weed biomass yields

Corn vs Soil Nitrogen

Correlation analyses showed that grain yield was positively correlated with SMNf, but not with SMNs. Corn grain C and N concentrations, stover N, and overall corn N uptake had significant moderately strong positive linear relationships with both SMNf and SMNs (r = 0.55 – 0.69; Table 3.6). Stepwise regression analysis showed that stover N concentration explained 52% of the variation in SMNf (Supplementary Table S1; Figure 3.11). Spring N was moderately related to grain C and N concentrations (R^2 = 0.59; Figure

3.12). Stover C concentration and yield were not significantly related to either SMNf or SMNs. The C/N ratios for both grain and stover were negatively correlated with SMNf and SMNs.

3.3.6.1. Soil Nitrogen vs Cover Crop Yield and Quality

There was a moderately strong positive linear relationship between SMNf and SMNs (Table 3.7). Fall CC biomass had weak negative linear relationships with both SMNf and SMNs. On the other hand, spring and total CC biomass had weak negative linear relationships with SMNf, but no significant relationships with SMNs. Cover crop tissue N concentrations did not have any significant relationships with fall weed biomass, SMNf or SMNs, whereas CC-C had significant, moderately strong, positive relationships with SMNf and SMNs. Cover cop C/N ratios were also weakly but positively correlated with SMNf, with no significant relationship with SMNs. The cumulative C and N from aboveground CC biomass were not significantly correlated with either SMNf or SMNs. Based on stepwise regression analyses, significant variations in total CC biomass (40%) and cumulative CC-C (40%) and N (48%) were explained by fall weed biomass (Supplementary Table S1).

Table 3.8. Pearson correlation analyses for cover crop yield and N and C concentrations with fall weed biomass, and residual soil mineral N in the fall (SMNf; n = 46), and spring (SMNs; n = 48)

	Fall weed biomass†	**SMNf**	**SMNs**
Fall aboveground biomass	0.45*‡	- 0.33*	- 0.33*
Spring aboveground biomass	0.68*	- 0.45*	- 0.23
Total aboveground biomass	0.65*	- 0.44*	- 0.27
Tissue N Content	-0.15	- 0.01	0.10
Tissue C Content	0.01	0.55*	0.58*
Cumulative aboveground biomass N	0.62*	- 0.27	- 0.13
Cumulative aboveground biomass C	0.65*	0.27	0.09
C/N ratio of aboveground biomass	0.12	0.35*	0.18
Fall weed biomass	–	-0.41*	-0.24
SMNf		–	0.65*

† Fall weeds represent the average of weeds sampled at grain harvest and at the end of the frost-free period.

‡ Significant Pearson correlation coefficients are indicated by an asterisk (*)

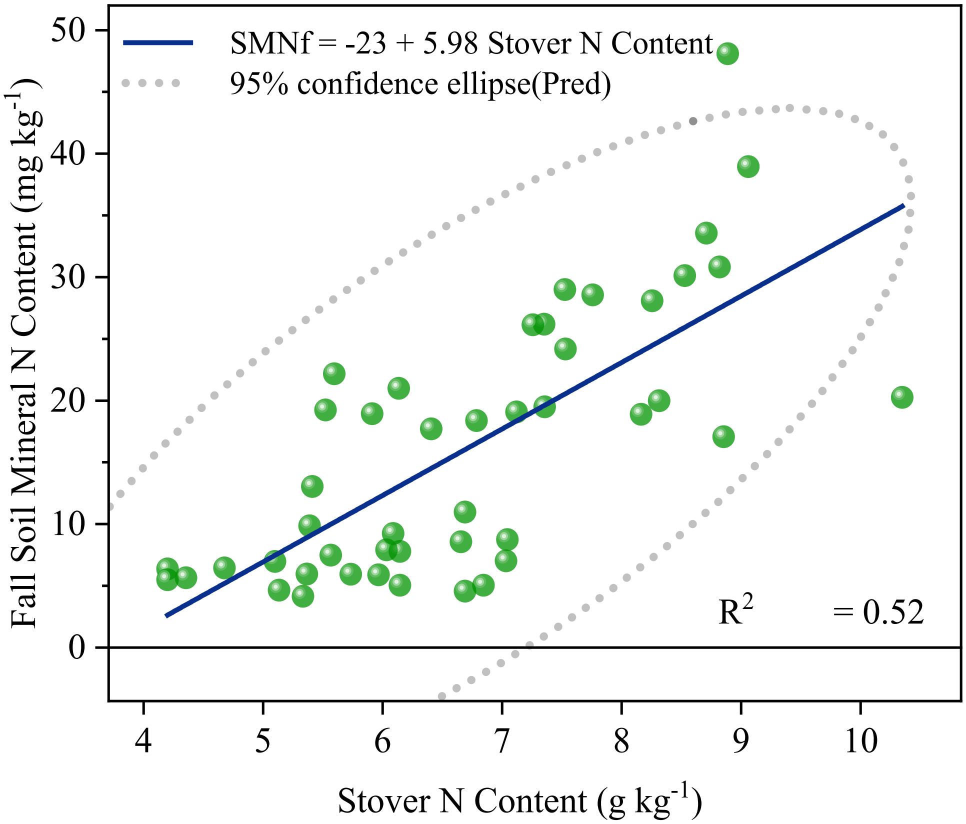


Figure 3.11. Relationship between stover N concentration and soil mineral N at corn grain harvest.

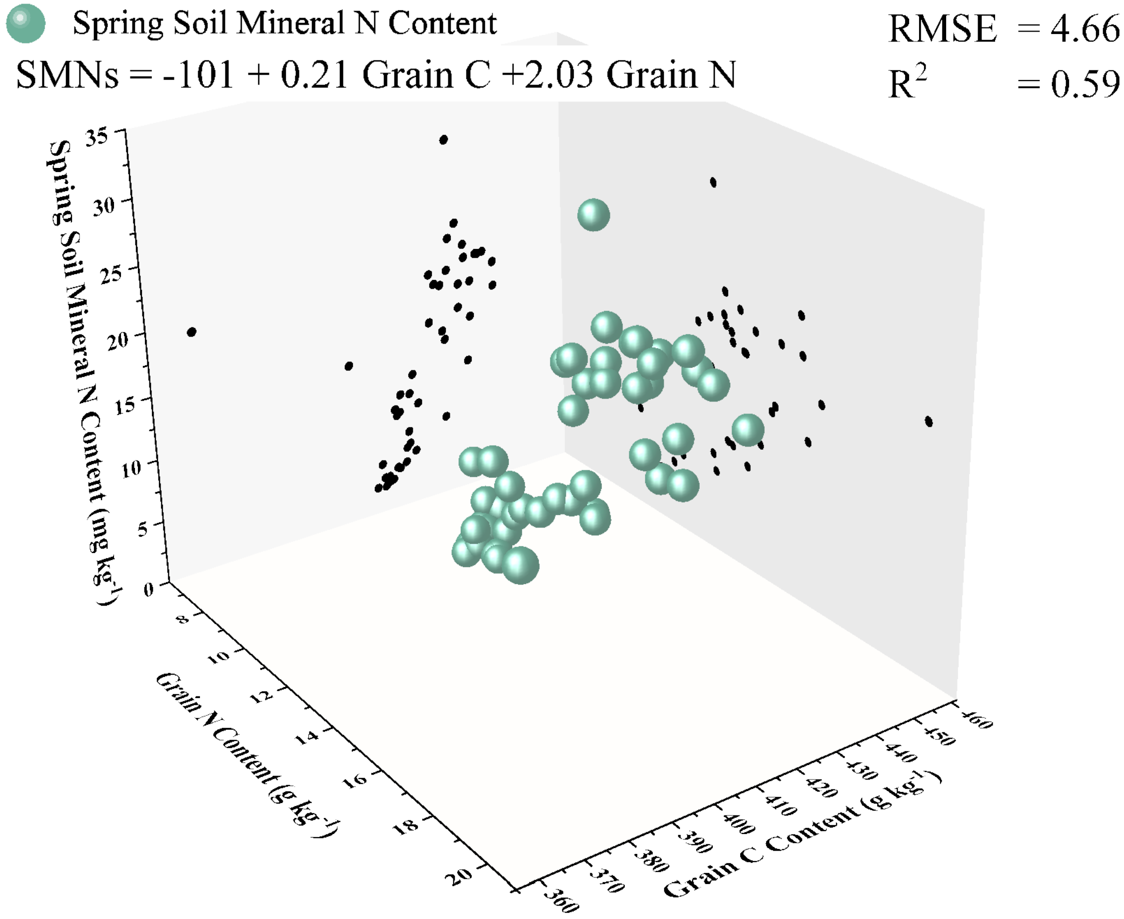


Figure 3.12. Soil mineral N concentration in the spring as a function corn grain C and N concentrations

3.4. Discussion

3.4.1. Corn and Cover Crop Yields

Most measured attributes were significantly different among site-years, and this was expected as crop or soil responses to cover cropping are mainly determined by variations in climate (Teixeira et al., 2016), soils, and management (e.g., seeding times, fertilizer management and corn varieties) (Alotaibi et al., 2018; Kablan et al., 2017; Moore and Mirsky, 2020). The main objectives of this study were to evaluate relationships between CCs, soil N and corn yield and N uptake. However, weed control was a glaring variable among the sites, and weed biomass became an inevitable confounding variable influencing most of the parameters of interest. The detrimental effects of weeds were clearly reflected at PTB15 where weed biomass was the highest and corn yield was significantly lower compared to ELR15. Prior to the start of these trials, the PTB15 site had been organically managed, hence the greater weed pressure. Also, planting was delayed, and there was a prolonged period of late-season water logging (October; Figure 3.4), as well as damage from deer, which led to substantial losses in yield. Grain yield and stover C concentration decreased with increases in weed biomass, consistent with studies that have shown that weed pressure can lead to economically detrimental yield losses; for e.g., a meta-analyses of studies from 21 US states and Ontario over a 7-yr period from 2007 to 2013 found weeds caused an average of 50% loss in grain corn yields (Soltani et al., 2016; Soltani et al., 2017).

Dry conditions were experienced across Ontario in 2016, and no significant yield differences were observed between ELR16 and RDG16. Also, the recorded corn grain yields and N uptake were higher than the preceding 2015 season. The grain yields recorded in this study are within the range of economic optimum yields of grain corn (Jarecki et al., 2018; Nyiraneza et al., 2010). Corn yields are known to be impacted by the timing of suboptimal conditions such as excess wetness (Mukhtar et al., 1990) or drought (Cakir, 2004). Corn is mostly sensitive to meteorological drought stress (moisture deficiency and heat stress) during the grain filling stage (Mishra and Cherkauer, 2010), so if conditions are favourable at that stage yields may be less negatively impacted. Greater corn N uptake in the 2016 compared to the wetter 2015 season may suggest that N uptake efficiency was improved under drier conditions, as leaching would also be minimal. The intermittent periods of drought in 2016 would have subjected the corn to periods of stress and may have triggered other mechanisms to enhance N acquisition. Such mechanisms include improvements in the root system, leaf structure, osmotic adjustment, and stomata regulation (Ilyas et al., 2021). Associations of corn with arbuscular mycorrhizal fungi (AMF) could increase corn drought tolerance due to transport of substantial amounts of nitrate through external AMF mycelium under drought conditions (Dodd, 2000; Subramanian and Charest, 1999). Although none of these mechanisms were investigated in this thesis, the results suggest that N uptake efficiency was not influenced by CCs but by site-year. However, greater residual SMN was in the hotter and drier season (2016), indicating the influence of drought on reducing N losses through leaching or denitrification (Bowles et al., 2018; Homyak et al., 2017).

The 2016 growing season was a very dry and hot season overall, especially at the time of CC seeding, and RC establishment was sparser than AR. In the 2015/16 season, there was significant CC biomass accumulation between grain harvest and the start of the frost period at PTB, but not at ELR (or RDG, where all CCs dried out; data not shown). This may have been mainly due to the complete removal of corn residues from the plots at PTB following harvest. Although the proportions of AR and RC biomass in the MIX treatment were not quantified, observations at sampling showed that AR dominated the MIX yields in the 2016-17 season. Visual and empirical observations throughout this study showed that the different climatic conditions within the six site-years (results from two were excluded due to CC establishment failure) disproportionately impacted RC compared to AR, leading to poor or failed RC establishment in the drier seasons. In 2016 when the RC establishment was limited, the AR in the MIX plots thrived and provided soil cover.

This indicates an advantage of growing CCs in mixtures, even one containing only two species. Growing CCs in grass-legume mixtures can lead to enhanced N acquisition (Nyfeler et al., 2011), and greater pest suppression (Cholette et al., 2018), while offsetting potential deficiencies in biomass accumulation (Chapagain et al., 2020), and enhancing the retention of excess N (White et al., 2017). Different CC species offer differential competitive abilities when conditions are sub-optimal, and this is important for ensuring success in realization of benefits.

Cover crop treatments in this study did not significantly reduce corn grain yields, regardless of their own highly variable biomass yields. The results of this study corroborate findings from previous studies in southern Ontario that found no yield penalties on grain corn from CCs (Belfry and Van Eerd, 2016; Coombs et al., 2017; Kuo and Jellum, 2000) or sweet corn (O'Reilly et al., 2012). This is also consistent with many other studies that reported non-significant corn yield penalties with interseeded AR or RC (Abdin et al., 1998; Brooker et al., 2020; Thilakarathna et al., 2015; Vyn et al., 2000; Vyn et al., 1999), even in cases with variable corn density (Baributsa et al., 2008). As of 2015, more than 4000 ha were interseeded with CCs in corn in the north-eastern US, Ontario and Quebec, without significant yield penalties overall (Groff, 2015). Almeida et al. (2018) reported that the yield and N nutrition (percentage N recovery efficiency) of grain corn interseeded on the same day with palisade grass was not significantly reduced. On the contrary, other studies have reported reductions in yields of crops succeeding CCs, for e.g., grain corn (Tollenaar et al., 1992; Tollenaar et al., 1993), forage (Malhi and Foster, 2011), silage corn (Raimbult et al., 1991) and spring wheat (Moyer and Blackshaw, 2009). Although interseeded CCs may not affect their ‘companion’ crop, they need to be managed carefully to avoid yield penalties for succeeding crops. In an assessment of residual CC effects on succeeding soybean yields in a study conducted by Clark (2018) at the same sites in this study, potential reductions in soybean yields following interseeded RC in grain corn were observed at ELR15.

3.4.2. Cover Crop Carbon and Nitrogen

Cover crops produced variable amounts of biomass as well as accumulating variable levels of C and N. The low N concentration of AR relative to RC is expected as RC can obtain additional N from biological N fixation. The values for C and N from RC biomass observed in thus study were within the range reported in other studies [119 kg N ha^{-1} (Yang et al., 2019); 68.8 – 145.9 N (Coombs et al., 2017); and 28 - 151 kg N ha^{-1} (Vaillancourt et al., 2017)]. The greatest cumulative CC biomass (and plant tissue C and N) was observed at PTB15. The greater total biomass at PTB15 could be partially attributed to greater exposure to light (Ballaré and Casal, 2000; Schipanski and Drinkwater, 2011) following corn residue removal. Also, there was delayed CC termination in the spring which allowed more time for biomass accumulation at PTB15. The AR and MIX treatments at the same site accumulated less C than RC, which may be reflective of the ability of RC to acquire N from BNF (Coombs et al., 2017; Vyn et al., 2000; Vyn et al., 1999). Red clover has been reported to potentially replace > 90 kg fertilizer N for the succeeding crop (Bruulsema and Christie, 1987). Liebman et al. (2012) reported a N fertilizer replacement value for corn of 87 – 184 kg N ha^{-1} for RC when used as a green manure. Others have reported lower apparent RC N credit [30 - 48 kg N ha^{-1}; (Gentry et al., 2013)]. For canola, a mix of red and white clovers had a mineral fertilizer replacement value of 22–82 kg N ha^{-1} (Vaillancourt et al., 2017).

For each CC, N concentrations were similar among site-years, suggesting relatively greater importance of genetics in determining N accumulation capacity compared to climatic or management differences e.g. different N fertilizer or glyphosate rates which could have impacted N availability and uptake (Sindelar et al., 2013). Red clover, being a legume, is distinctly different from AR, a cereal, and their inherent differences translate to the differences in their physiological and functional traits. This diversity is important as it may dictate the ESs provided by CCs more than biomass levels (Finney et al., 2016). The inherent ability of RC to accumulate N through BNF (McKenna et al., 2018) may have been large enough to overcome the vast differences in soil and environmental conditions at the different site years. The C/N ratios for MIX at ELR15 (11.8) and ELR16 (18.1) were identical to C/N ratios of RC (11.8) and AR (18.2), respectively. These seemed to reflect the predominant species that were competitive each season in the mixtures. Although we did not determine species proportions in the mixture, visual observations also confirmed that RC predominated the MIX treatments at ELR15 and PTB15. Similarly, AR predominated the mixtures at ELR16 and RDG16. Cover crop C/N ratios are more important for succeeding crops than they are for the companion crop (Kuo and Jellum, 2000). White et al. (2017) reported decreases in grain corn yield (succeeding crop) with increases in CC C/N ratios, and positive correlations between corn yield and spring CC biomass N concentration. In this study, the CC C/N ratio was only weakly correlated with grain N concentration and grain C/N ratio, and this relationship did not yield a significant linear relationship with regression analysis.

3.4.3. Relationships Between Corn, Cover Crop and Soil

Due to the high variability in climatic conditions and soils at our different sites, as shown by highly significant site-year effects on most response variables, correlation and regression analyses showed weak to moderate relationships among variables. Despite variations in management between PTB15 and the other site-years, results indicate a greater importance of climatic factors in determining relationships between corn yield or quality with CC biomass (Teixeira et al., 2016). Corn grain yield, grain and stover C, and corn N uptake had weak and moderately strong negative correlations with fall CC biomass. These relationships did not translate into detectable effects of the CCs on the corn attributes in the global ANOVA. However, regression analyses showed that grain and stover C contents explained more than 50% of the variation in fall CC biomass. This may be due to the influence of corn canopy cover on interseeded cover crops which reduces growth of CCs through light interference (Youngerman et al., 2018). At corn seeding densities above 75000, Baributsa et al. (2008) noted that corn can reduce interseeded CC dry matter yields, as is the case with the seeding density of 78000 seeds ha^{-1} in the current study.

Residual mineral N in the fall had a weak correlation with fall CC biomass and was not significantly influenced by fall CC biomass based on regression analysis results. Spring CC biomass was significantly influenced by fall CC biomass and fall weed biomass SMNf, SMNs, and CC biomass and C accumulation were significantly correlated with corn grain yield and stover N. No significant association of soil N with CC N accumulation was detected in this study, which is contrary to other studies reporting significant reductions in SMN due to CCs (Dabney et al., 2010; Karlen et al., 2019; McKenna et al., 2018). Coombs et al. (2017) reported that SMNs was 10 kg N ha^{-1} lower with legume CC treatments compared to no cover treatments following grain corn in ON. Their study differs from the current study in that legume CC establishment was very limited at most site years except PTB15. A meta-analysis in the Pampas with 67 field experiments showed average decreases in soil NO_3^--N of 30% for both legumes and non-legumes (Alvarez et al., 2017), the main factor influencing these results being the establishment of cover crops in the systems. Harris et al. (1994) found more RC N (47% of total inputs in residues) retained in the soil compared to fertilizer N but was not observed in this current study. The results of the current study suggest that the amount of CC biomass accumulated at the start of the frost period was not sufficient to influence soil N status in the spring. Spring mineral N in this study was measured only in the 0 – 15 cm depth as unincorporated CC residues have greater influence on soil N in the topmost layer Veras et al. (2016). It is possible that some SMN may have leached beyond the 15 cm depth from which spring SMN was measured. However, no measurements of N losses through leaching or denitrification were done in this current study, so it I am unable to conclusively attribute these findings to these processes.

Cover crop N concentration varied by CC type and was neither affected by site-year nor correlated with soil or corn attributes. This indicates the relative importance of CC genetics in influencing N accumulation (Kuo and Jellum, 2000). Greater residual SMN (SMNf or SMNs) was associated with greater total CC-C according to correlation analysis. This suggests that CC-C is potentially more responsive to soil N status than CC-N concentration. The lack of relationship of soil N with CC-N may also explain why CCs in this study did not significantly affect corn N uptake i.e., CC N accumulation did not influence N supply for corn or residual SMN following corn harvest. In fact, there was a positive relationship between residual N and the C/N ratio of CCs; CCs with high C/N ratios were associated with plots with higher SMNf, suggesting that soil with more N led to greater CC biomass C accumulation.

3.5. Conclusions

Interseeded AR, RC, or their mixture accumulated 0.45 – 1.6 Mg ha^{-1} total biomass before and after winterkill. No CCs significantly affected corn grain or stover yields, N uptake, or residual SMN measured at corn harvest or the following spring. These results are not surprising for a system that is dominated by corn residues relative to the meager amount of CC biomass accumulated at the time of corn harvest. Correlation analysis suggests CC establishment (biomass accumulation) was significantly influenced by corn stover and grain C contents. The results suggest that interseeded AR and RC have low risk of interfering with grain corn yields or quality, therefore can be adopted without yield penalties. However, CC establishment may be limited in systems with higher corn seeding densities or if introduced after corn canopy closure. Also, future research could assess the potential benefits of fertilizing CCs to promote biomass accumulation. Cover crops also reduced weeds by more than 50%, showing great potential for producers who wish to reduce weed pressure and ultimately herbicide use.

4. RESPONSES OF BIOLOGICAL SOIL HEALTH INDICATORS TO INTERSEEDED ANNUAL RYEGRASS AND RED CLOVER IN CORN

4.1. Introduction

Soil is critical for survival and support of all terrestrial organisms, as it provides a habitat for macro- and microorganisms, and a rooting medium from which plants obtain all their nutrients except C. Soil organic matter is a key attribute of soil health, and most soil biological and physicochemical processes are directly influenced by SOM, for e.g., nutrient cycling, aggregation, water movement, moisture status and aeration (Benitez et al., 2016). While SOM is generally the single most robust indicator of soil health because it is highly correlated with all other soil health attributes, measurable changes in total SOM are only detectable long after initiation of changes to management [>10 yr. (McDaniel et al., 2014b)]. Total SOM is composed of different pools at variable levels of decomposition or stabilization, and these range from freshly added residues or organic material to highly recalcitrant forms (Janzen et al., 1997; Poirier et al., 2005; Wander, 2004). Labile or biologically active SOM pools are highly responsive to environmental and management changes and have quick turnover times. Due to their dynamic nature and high correlation with total SOM, labile SOM fractions can be used as early indicators of potential long-term changes in SOM (Franzluebbers and Stuedemann, 2008).

Labile SOM includes POM, light fraction SOM, active C (POXC), PMN, and soil microbial biomass (SMB) (Agomoh et al., 2020; Das and Varma, 2011; Obalum et al., 2017; Powlson et al., 1987). Particulate SOM comprises the organic C and N content of primary soil particles in the 53-2000 μm size class and is reported as one of the most sensitive indicators of soil health (Duval et al., 2018). Soil microbial biomass is a measure of the total pool of soil microorganisms. During decomposition, soil microbes use enzymes to breakdown SOM and release nutrients into the soil as they transform SOM into increasingly stable forms. Soil microbial biomass is therefore highly dependent on and positively correlated with SOM content and quality (Blaud et al., 2014). Furthermore, measurements of microbial activity and functions, e.g., respiration and enzyme activities, as well as microbial community diversity and structure, can be useful in evaluating soil microbial responses to environmental and management changes.

Microbial respiration is a direct indicator of microbial activity (Franzluebbers, 2016; Franzluebbers and Veum, 2020), while soil enzymes are highly correlated with the amount and quality of organic matter added (Geisseler et al., 2011; Sinsabaugh et al., 2008). β-glucosidases are key enzymes in C-cycling (Sinsabaugh et al., 2008); dehydrogenases are oxidative enzymes present in all microorganisms and only associated with active bacterial cells, and their activity also reflects microbial metabolic activity (Dotaniya et al., 2019; Utobo and Tewari, 2015). Community-level physiological profiling (CLPP) is a technique used to infer microbial community structure based on changes in the utilization patterns of different C sources (Garland and Mills, 1991; Weber and Legge, 2010). It can be used to compare microbial community diversity as it relates to CCs.

The main objective of this study was to evaluate short-term responses of dynamic soil attributes to the inclusion of AR and RC as interseeded cover crops in corn-soybean systems in southern Ontario. The interseeding of cover crops into standing corn is a relatively new strategy in Ontario, and a lot is still unknown about the scale of effects on soil biological parameters. The well-known benefits of CCs are the stabilization and protection of topsoil from water and wind erosion through increasing the abundance of above- and belowground biomass (Mervin and McLarty, 2017). Moreover, through living root exudates, dead root biomass and senesced leaf litter, CCs can contribute significant amounts of SOM-C and -N to the soil system (Dennis et al., 2010; Høgh-Jensen and Schjoerring, 2001; Wittwer et al., 2017). There are no current studies that have evaluated seasonal soil health effects of CCs interseeded into standing grain corn. The hypothesis of this study was that interseeded AR and RC could significantly increase dynamic soil health parameters such as SMB, POM, soil respiration, enzyme activities and microbial community structure at the end of the first CC cycle. If true, these soil health attributes could potentially be used in the assessment of these and other CC species' use for soil health provisioning in different cropping systems and regions.

4.2. Materials and Methods

4.2.1. Experimental Design and Treatment Layout

Detailed descriptions of the sites, CC treatments and agronomic practises are provided in Sections 3.2.1 and 3.2.2. Annual ryegrass, RC, and MIX were seeded into corn at the V4-V6 stage and terminated the following spring, followed by soil sample collection for the assessment of changes in soil health attributes. In 2015, soils samples were collected at grain harvest, and the site-years are hereby designated PTB15 and ELR15. The following spring soil samples were collected from the same plots after CC termination, and the site-years are referred to as PTB16 and ELR16. For the 2016/17 season, samples were only collected in the spring, and the site-years are ELR17 and RDG17.

4.2.2. Soil Sampling

Soil samples for analyses of soil health parameters were collected from the 0 - 15 cm depth. At each soil sampling event, nine cores were collected from the row spaces between the 9 central cover crop rows (Figure 3.2). The cores were chosen in a zig-zag pattern from anywhere within the sampling area (regardless of where cover crops were established), placed into polyethylene bags and kept cool in coolers until they were brought back to the lab for analyses. Samples for SMB, soil basal respiration (SBR), and β-glucosidase or dehydrogenase activities (BGA and DHA, respectively) were preserved at 4°C and analyzed within 7 d. Samples for POM, flush of CO_2 upon rewetting, and CLPP analyses were air-dried until analyses.

4.2.3. Laboratory Analyses

Particulate Organic Matter

Particulate organic matter (POM; 53 – 2000 μm diam.) was determined following a procedure modified from the method of Moni et al. (2012). Briefly, 25 g of air-dried pre-sieved soil (<2mm) and 60 glass beads (5 mm diam.) was shaken for 16 h at 130 rpm with 100 mL water. After shaking, the suspension was poured onto a < 2 mm sieve overlaying a <53 μm sieve to separate the beads and POM residues. The residue collected on the <53 μm sieve was then dried in an oven at 60°C for 48 h and pulverized using a Retsch Planetary ball mill PM100 (Verder Scientific Inc., Newtown, PA). The POM-C and -N were determined by dry combustion on a Vario MAX cube CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Water-Filled Pore Space

To calculate water-filled pore space (WFPS), a preliminary test was conducted to determine the total volume and bulk density of packed soil. For this, three 500-mL Mason jars were filled with 200-g soil and moistened with tap water to achieve ≈30% gravimetric moisture content (based on observed field moisture content at sampling). This initial moisture was necessary to facilitate packing of soil before measuring total volume and/or bulk density. Packing was done by tapping the jars five times to settle the moistened soil. Following this, a second set of 500-mL Mason jars were placed beside the first three. Water was added to the empty jars, up to the levels of the settled soil. The volume of the water was recorded to denote the total volume of the 200 g of packed soil. Bulk density was calculated based on the measured total volume and oven-dry weight of the soil. Total porosity was calculated as the difference between the total volume and the volume of solids. Percentage WFPS was calculated as a fraction of this total porosity value as shown in Equation 2.

$$\text{WFPS} = \frac{V_W}{V_T - V_S} \times 100 \qquad [2]$$

where WFPS is water-filled pore space; V_W, V_T, and V_S are the water-, total-, and solid -volume, respectively. (V_T - V_S) is the total pore volume.

Soil Basal Respiration

Soil basal respiration was determined within four days following sampling using a slightly modified procedure to that used by Dinesh et al. (2003). For this, 100 g of sieved (<2 mm) field moist soil was placed into 1-L mason jars and pre-incubated for 3 d at 25°C, and 50% WFPS was considered the optimal moisture content for microbial activity in this study (Linn and Doran, 1984). A small container with 50 mL of 0.025 M NaOH was placed in each Mason jar to trap evolved CO_2, and the jars were tightly capped and left for 24 h before collecting the traps. Following trap retrieval, 2 ml 1 M $BaCl_2$ were added to precipitate all the carbonate. The amount of unreacted NaOH was immediately measured by back-titration with 0.01M H_2SO_4 using an autotitrator (PC-Titrate, ManTech Inc., Guelph, ON).

Soil Microbial Biomass

Soil microbial biomass was determined using the chloroform fumigation-extraction procedure according to Voroney et al. (2008). Twenty-five grams (dry wt.) of the pre-incubated samples used for SBR were fumigated in a desiccator with ~10 mL of ethanol-free chloroform. Total organic C and N in fumigated and unfumigated samples was extracted with 75 mL of 0.5 M K_2SO_4. Total dissolved organic C (DOC) in the extracts was determined on a TOC analyzer (TOC-VCPH, Shimadzu Scientific Instruments Inc., Columbia, MD). Total N was determined by flow-injection analysis (Bran Luebbe AA3, Seal Analytical Inc., Mequon, WS) following digestion with $K_2S_2O_8$. The SMB-C and SMB-N Were calculated as the difference between fumigated and unfumigated samples according to the formula:

$$\text{SMB-C} = \frac{C_f - C_{uf}}{k_{ec}} \qquad [3]$$

where C_f and C_{uf} are amounts of C in fumigated or unfumigated samples, respectively; $k_{ec} = 0.35$. The same formula was used to calculate SMB-N, except a k_{en} value of 0.50 was used instead. The k_{ec} and k_{en} values represent the efficiency of extracting C or N, respectively.

Flush of CO_2 upon rewetting of air-dried soil

The flush of CO_2 upon rewetting of air-dried soil (fCO_2) was determined as described by Franzluebbers (2018). Briefly, 100g of air-dry soil was moistened to 50% WFPS in 2-L Mason jars. Small beakers containing 50 mL of 0.025 M NaOH were placed in the Mason jar and the lids were tightly capped. After 3 d, the NaOH traps were retrieved, and the trapped CO_2 was measured as described in Section 0 above for SBR.

β-Glucosidase Activity

The cellulolytic activity of the soil was determined by measuring BGA using p-Nitrophenyl-β-D-glucopyranoside (20 mM) as a substrate (Eivazi and Tabatabai, 1988; Geisseler and Horwath, 2009). Duplicate 1 g pre-incubated (7 d at 50% WFPS) soil samples were treated with 0.25 mL of toluene and 2.5 mL sodium acetate buffer (0.2 M, pH 6.0) in a 50-mL Erlenmeyer flask. The flasks were stoppered and shaken for 1 h at room temperature. After shaking, 1 mL of 0.5 M $CaCI_2$ and 4 mL of 0.1 M Tris buffer (pH 12) were added. The p-nitrophenol product was determined on a microplate reader based on the standard curve of known concentrations of p-nitrophenol standards at 430 nm (Biotek Instruments Inc., Winooski, VT).

Dehydrogenase Activity

The dehydrogenase activity, which reflects the oxidative capacity of the soil, was determined using the method described by Von Mersi and Schinner (1991). Briefly, duplicate 1 g (dry wt.) samples of pre-incubated (7 d) moist soil were mixed with 1.5 mL of 1 M Tris buffer (pH 7), and 2 mL iodonitrotetrazolium chloride (INT) (10 mg mL^{-1}) in foil-wrapped 15 mL centrifuge tubes. The suspension was shaken at ~200 rpm for 24 h, immediately followed by addition of 10 mL of a 1:1 N,N-dimethylformamide/ethanol extractant to stop the reaction. The suspension was shaken for 1 h. Approx. 1.5 mL aliquots were centrifuged at 4,300 rpm for 10 min. A standard curve was created by measuring the absorbance of iodonitrotetrazolium formazan standards at a concentration range of 0 – 27 μg mL^{-1} extractant at 464 nm on a Biotek™ Epoch™ Microplate Spectrophotometer (Biotek Instruments Inc., Winooski, VT).

Community-level physiological profiling

Community-level physiological profiles of soil were determined by measuring the color development due to the reduction of tetrazolium violet during the respiration of 31 sole C sources on 96-well BIOLOG Ecoplates™ (BIOLOG Inc., California). Forty-five milliliters of autoclave-sterilized 0.85% NaCl solution were added to 5 g soil that had been pre-incubated for seven days at 50% WFPS. After shaking for 60 min, 10 mL of the suspension was centrifuged at 25 x g (420 rpm) for 3 min (Centrifuge 5702; Eppendorf AG, Hauppauge, NY). The supernatant was diluted to 10^{-3}, and a 150μL aliquot was pipetted into each of the 96 wells of the EcoPlate (Figure 4.1). All plates were incubated at 25 °C and well optical density (OD; absorbance at 590nm) was measured using an Epoch spectrophotometer (Biotek Instruments Inc., Winooski, VT) once every 24 h for 9 d. Data from the 96 h time point was used because absorbance readings exceeded 2 beyond this point (outside the linear absorbance range) (Weber and Legge, 2010).

4.3. Statistical Analyses

Analyses of Variance

The effects of site-years and CCs on soil health parameters were evaluated using two-way ANOVA using a general linearized mixed model (PROC GLIMMIX) in SAS v. 9.4 (SAS Institute Inc., 2019). Effects of site-years, CCs and their interactions were modelled as fixed factors, whereas the blocks/replicates and their interactions with main effects were considered as random effects. Before accepting ANOVA results, a Shapiro Wilk's test was used to ensure assumptions of normality and homogeneity of variance were met using PROC UNIVARIATE. Least squares mean estimates were compared using the Tukey-Kramer multiple means comparison procedure at 5% significance level for all pairwise comparisons.

Analyses of BIOLOG Data

Overall microbial activity in each microplate, expressed as average well-color development (AWCD), was calculated as shown in Equation (Garland and Mills, 1991). The AWCD values were subjected to an ANOVA, with cover crop treatments and site-years as fixed effects, while blocks and their interactions with fixed effects were random effects.

$$\mathrm{AWCD} = \Sigma(\mathrm{OD}_i - \mathrm{R})/31 \qquad [4]$$

where OD_i is the optical density (OD) value for each C substrate well and R is the average OD from the control wells.

To evaluate the influence of different CC treatments on microbial utilization of different C sources, principal component analysis (PCA) was performed on standardized OD data (Equation 5) using PROC FACTOR in SAS. Standardization was done in order to eliminate the effects of different inoculum densities between plates (Calbrix et al., 2005; Graham and Haynes (2005). Standardized OD values that were negative or < 0.06 were set to zero.

$$\text{Standardized OD} = (\text{OD}_i - \text{R})/\text{AWCD} \qquad [5]$$

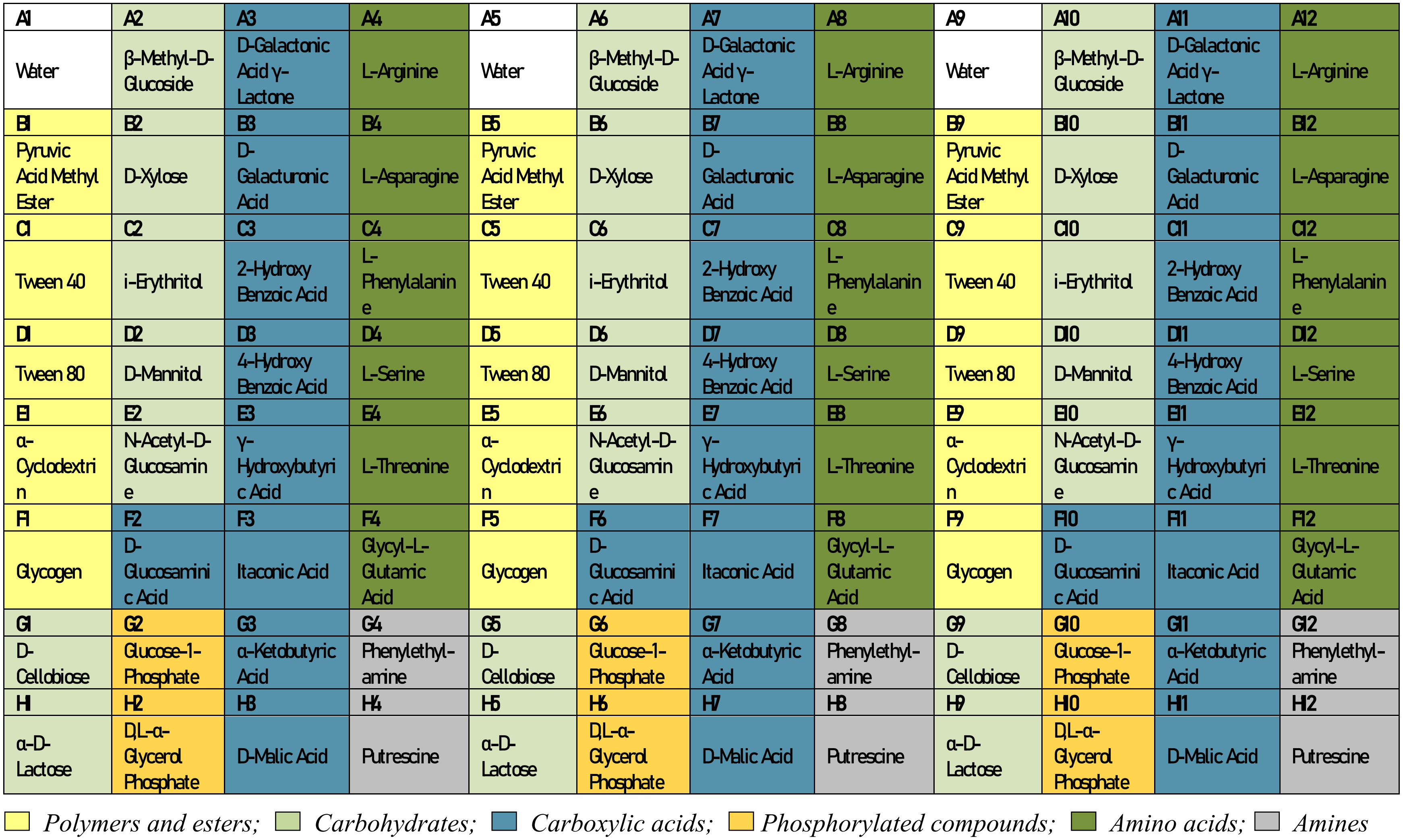
The varimax rotation (an orthogonal rotation which results in uncorrelated components) was applied to improve the interpretability of results (O'Rourke and Hatcher, 2013). Components with eigenvalues > 1 were retained. Next, the rotated factor solution was assessed to identify variables that had significant loadings (correlations >60%) on each PC. Variables that loaded on only one PC were retained and the rest were eliminated. After eliminating non-significant variables, the PCA was re-run to obtain a final solution.

4.4. Results

4.4.1. Responses of Soil Biological Parameters to Cover Crops

There were no significant effects of CCs on POM, SMB, respiration, or enzyme activities at 5% significance (Table 4.1). However, there was a significant trend ($p = 0.08$) which showed that POM-C increased in the order NOCC<MIX<RC<AR. There was a significant site-year effect on all measured parameters. In the fall and spring of the 2015-2016 growing season, POM-C, POM-CN, SMB-C, and SBR were significantly lower in soil samples from ELR16 compared to those from PTB16 (Table 4.1). In contrast, POM-N was significantly greater at ELR16, whereas SMB-N and SMB-CN were not significantly

different between the two site-years. There were no significant differences in fCO_2 between the two sites. B-glucosidase activity was significantly lower at ELR16 than at PTB16 in both fall and spring samples (Table 4.2). On the other hand, no significant differences in DHA were observed between the two sites in the fall, but there was lower DHA at ELR16 than at PTB16 in spring samples. In the 2016-2017 growing season, only spring samples were collected from ELR17 and RDG17. POM-C was significantly lower at ELR17 than at RDG17, whereas POM-N, SMB-N, BGA, and DHA were greater at Elora than at Ridgetown. There were no significant differences in SMB-C, SMB-CN, and fCO_2 between the two sites.



A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A5 Water	A6 β-Methyl-D-Glucoside	A7 D-Galactonic Acid γ-Lactone	A8 L-Arginine	A9 Water	A10 β-Methyl-D-Glucoside	A11 D-Galactonic Acid γ-Lactone	A12 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B5 Pyruvic Acid Methyl Ester	B6 D-Xylose	B7 D-Galacturonic Acid	B8 L-Asparagine	B9 Pyruvic Acid Methyl Ester	B10 D-Xylose	B11 D-Galacturonic Acid	B12 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C5 Tween 40	C6 i-Erythritol	C7 2-Hydroxy Benzoic Acid	C8 L-Phenylalanine	C9 Tween 40	C10 i-Erythritol	C11 2-Hydroxy Benzoic Acid	C12 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D5 Tween 80	D6 D-Mannitol	D7 4-Hydroxy Benzoic Acid	D8 L-Serine	D9 Tween 80	D10 D-Mannitol	D11 4-Hydroxy Benzoic Acid	D12 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E5 α-Cyclodextrin	E6 N-Acetyl-D-Glucosamine	E7 γ-Hydroxybutyric Acid	E8 L-Threonine	E9 α-Cyclodextrin	E10 N-Acetyl-D-Glucosamine	E11 γ-Hydroxybutyric Acid	E12 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F5 Glycogen	F6 D-Glucosaminic Acid	F7 Itaconic Acid	F8 Glycyl-L-Glutamic Acid	F9 Glycogen	F10 D-Glucosaminic Acid	F11 Itaconic Acid	F12 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G5 D-Cellobiose	G6 Glucose-1-Phosphate	G7 α-Ketobutyric Acid	G8 Phenylethyl-amine	G9 D-Cellobiose	G10 Glucose-1-Phosphate	G11 α-Ketobutyric Acid	G12 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H5 α-D-Lactose	H6 D,L-α-Glycerol Phosphate	H7 D-Malic Acid	H8 Putrescine	H9 α-D-Lactose	H10 D,L-α-Glycerol Phosphate	H11 D-Malic Acid	H12 Putrescine

🟨 *Polymers and esters;* 🟩 *Carbohydrates;* 🟦 *Carboxylic acids;* 🟧 *Phosphorylated compounds;* 🟩 *Amino acids;* ⬜ *Amines*

Figure 4.1. Layout of 31 C sources on BIOLOG EcoPlates™ used to assess soil microbial community structure (Zak et al., 1994)

Table 4.1. Analysis of variance results for the effects of site-year and cover crops on soil particulate organic matter, microbial biomass, and respiration.

Source of Variation	**Particulate Organic Matter**			**Soil Microbial Biomass**			**Soil Basal Respiration**	**fCO2**
	C	**N**	**C/N Ratio**	**C**	**N**	**C/N Ratio**		
	g/kg			mg kg soil^{-1}			μg CO_2-C g soil^{-1} h^{-1}	μg CO_2-C g soil^{-1} 3d^{-1}
Site-Year (SY)								
2015 Fall								
Elora	17.5 b	0.685 a	26.0 b	84.0 c	48.9 bc	1.75 b	0.89 c	-
Peterborough	22.5 a	0.478 b	49.0 a	140 b	61.3 ab	2.31 b	1.52 a	-
2016 Spring								
Elora	13.7 c	0.594 a	23.2 b	117 bc	60.4 ab	3.11 b	1.23 b	143 a
Peterborough	18.9 b	0.475 b	41.2 a	196 a	85.3 a	3.05 b	1.47 a	138 a
2017 Spring								
Elora	14.0 c	0.624 a	22.5 b	142 b	15.3 c	12.6 a	-	66.1 b
Ridgetown	6.81 d	0.350 c	19.5 b	137 b	90.1 a	2.49 b	-	88.5 b
Standard Error	*0.924*	*0.021*	*2.52*	*11.1*	*8.11*	*1.44*	*0.05*	*8.77*
Cover Crop (CC)								
No cover	14.5	0.52	28.5	125	54.7	5.11	1.22	114
Annual ryegrass	16.7	0.544	31.9	141	64.5	4.62	1.32	106
Red clover	16.1	0.549	30.8	140	56.3	3.68	1.3	109
Mixture	15.1	0.525	29.7	138	65.4	3.48	1.25	108
Standard Error	*0.807*	*0.017*	*2.16*	*8.99*	*6.72*	*1.22*	*0.05*	*8.77*
	p-value							
SY	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CC	0.08	0.655	0.56	0.59	0.541	0.679	0.433	0.9
SY × CC	0.845	0.824	0.895	0.981	0.99	0.952	0.419	0.5

Table 4.2. ANOVA results of cover crop effects on soil enzyme and bacterial metabolic activities at three sites in Ontario

Source of Variation	**Enzyme Activity**		**AWCD**
	Betaglucosidase	**Dehydrogenase**	
	μg PNP g^{-1} h^{-1}†	μg INTF g^{-1} h^{-1}	
Site-Year (SY)			
2015 Fall			
Elora	276 d	69.2 a	
Peterborough	354 c	71.8 a	
2016 Spring			
Elora	441 b	30.3 d	0.463
Peterborough	567 a	41.0 c	0.347
2017 Spring			
Elora	488 b	57.9 b	0.111
Ridgetown	205 e	46.8 c	0.209
Standard Error	*18.8*	*2.58*	*0.107*
Cover (CC)			
No cover	394	53.2	0.120 b
Annual ryegrass	389	53.2	0.546 a
Mixture	383	55.3	0.281 b
Red clover	389	49.7	0.182 b
Standard Error	*16.5*	*2.58*	*0.107*
	P-value		
SY	< 0.001	< 0.001	0.139
CC	0.940	0.197	0.044
SY × CC	0.327	0.829	0.978

† PNP, p-nitrophenol; INTF, iodonitrotetrazolium formazan

‡ AWCD values calculated from OD_{590} readings after 96 h of incubation.

4.4.2. Effects of Cover Crops on Soil Metabolic Activity

There was a significant CC treatment effect on microbial metabolic activity at 96 h of BIOLOG EcoPlate incubation (AWCD; Table 4.2). Averaged for all site-years, the 96-h AWCD for AR was significantly greater than that of RC, MIX, or NOCC (Figure 4.2).

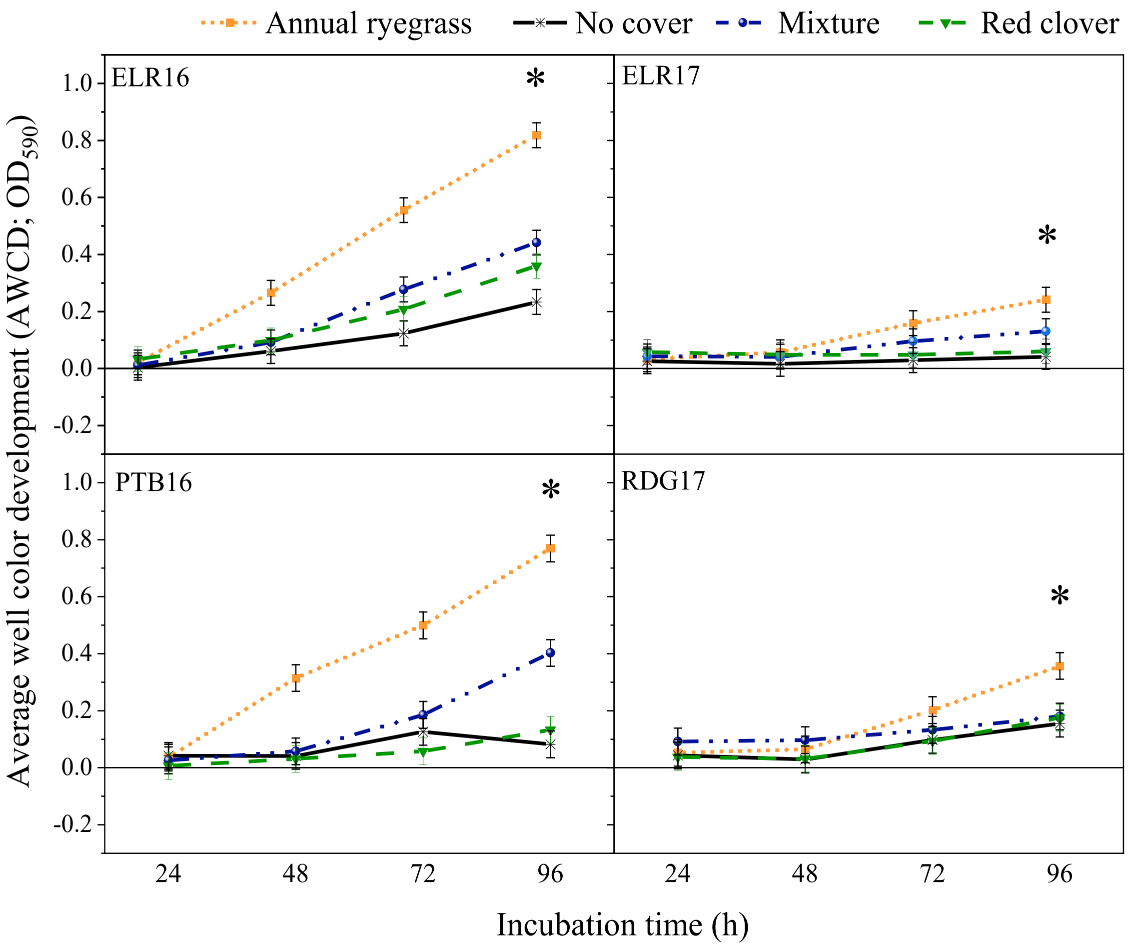


Figure 4.2. Average well color development (AWCD) following 96-h incubation of soil samples from plots in which annual ryegrass, red clover, or their mixture were interseeded into standing corn in Southern Ontario in the 2015/16 and 2016/17 corn growing seasons. The asterisk denotes significant differences.

4.4.3. Community-Level Physiological Profiles

To assess microbial community diversity, transformed absorbance data (standardized OD) from BIOLOG EcoPlates™ were analyzed using PCA. Standardized OD values ranged between 0 and 3, with those < 1 indicating color responses smaller than the AWCD and those > 1 being greater color responses than the AWCD (Supplementary Figure).

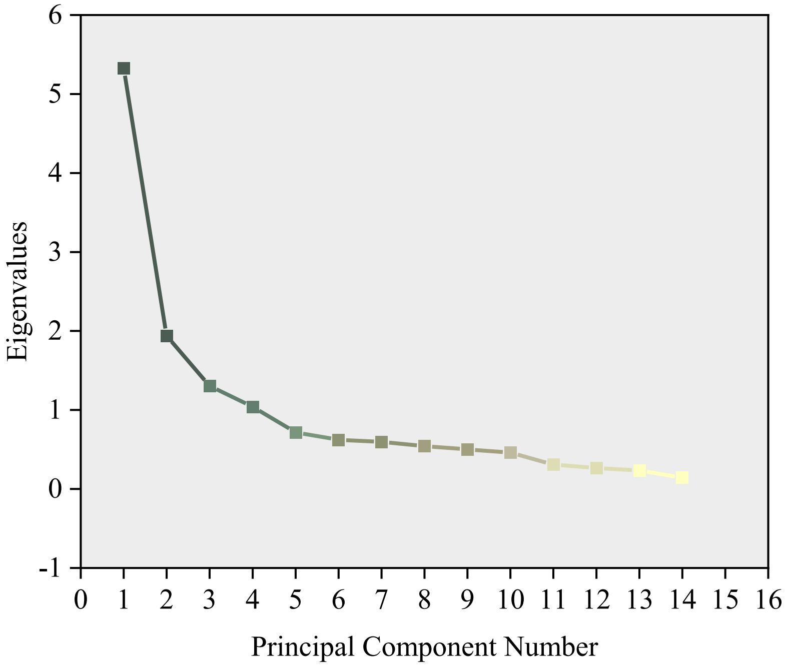


Figure 4.3. Scree plot of eigenvalues of PCs obtained from the analysis of the effects of cover crops on metabolism of 17 C sources.

Table 4.3. Eigenvectors of BIOLOG Ecoplate™ substrates loaded to the first four principal components, including eigenvalues and variance explained by each PC.

Guild	**Substrate**	**ID**	**PC1**	**PC2**	**PC3**	**PC4**
Carboxylic acids	Itaconic Acid	ITA	**0.34**	0.13	-0.03	-0.30
Polymers/Esters	Pyruvic Acid Methyl Ester	PAM	**0.33**	0.19	-0.24	-0.03
Carbohydrates	D-Cellobiose	DC	**0.31**	0.00	0.06	-0.20
Carboxylic acids	D-Malic Acid	DMA	**0.31**	0.23	-0.10	-0.25
Carbohydrates	α-D-Lactose	AD	**0.30**	0.04	-0.14	-0.26
Carbohydrates	D-Mannitol	DML	**0.30**	0.17	-0.07	-0.18
Amino acids	L-Arginine	LAG	0.30	**-0.37**	0.17	0.15
Amino acids	L-Serine	LS	0.30	**-0.42**	-0.08	0.18
Amines/amides	Phenylethyl-amine	PA	0.28	**-0.40**	-0.14	0.20
Carbohydrates	D-Xylose	DX	0.24	**-0.22**	0.13	0.25
Polymer/Ester	Glycogen	GN	0.19	0.24	**0.55**	0.08
Ketonic acids	α-Ketobutyric Acid	AKA	0.16	0.18	**0.66**	0.14
Amino acids	Glycyl-L-Glutamic Acid	GGA	0.13	0.34	-0.21	**0.50**
Amino acids	L-Threonine	LT	0.13	0.37	-0.24	**0.52**
Eigenvalue			5.33	1.94	1.30	1.04
Proportion of variance (%)			38.1	13.8	9.32	7.43
Cumulative variance (%)			38.1	51.9	61.2	68.6

† Bold-faced eigenvectors had significant correlations (Pearson's r > 0.6) with corresponding PCs.

Four PCs were retained based on the eigenvalue-one criterion, the scree plot (Figure 4.3), and analyses of rotated factor solutions. Seventeen variables which either correlated with more than one PC or failed to load (r>0.60) in any PC (O'Rourke and Hatcher, 2013) were eliminated. PCs 1 – 4 explained 68.6% of the total variation in BIOLOG Ecoplates™ data (Table 4.3). PC1 explained 38.1% of the total variance, and substrates with significant loadings were two carboxylic acids, three carbohydrates and one ester (all with positively correlated eigenvectors ≥0.30). The second PC contained two amino acids [LA (L-arginine) and LS (L-serine)], an amine (PA, phenylethyl-amine), and a carbohydrate (DX; D-xylose). PC3 comprised of one polymer (glycogen) and one ketonic acid (α-Ketobutyric Acid) while PC4 had two amino acids (L-Threonine and Glycyl-L-Glutamic Acid).

The loading patterns of the 14 C-sources showed that the level of utilizations of all substrates were positively correlated with PC1 (Figure 4.4, Supplementary Figure S2). The corresponding score plots of the site-year and CC treatments (Figure 4.4, Supplementary Figure S3) show no clear separation amongst treatments. The scores for all CCs from ELR17 and RDG17 were not associated with the substrates with the most significant loadings. However, AR and MIX treatments from PTB16 and ELR17 were more widespread and closely associated with the substrates with significant loadings.

4.5. Discussion

In this study, the short-term responses of soil health parameters to interseeded AR, RC and MIX were evaluated. Results showed that all measured parameters, except for AWCD (bacterial metabolic activity) and CLPPs measured using BIOLOG EcoPlates™, did not significantly respond to CCs in any of the four site-years. These results are consistent with other short- and medium-term studies involving these parameters (Ladoni et al., 2016; Liebig et al., 2015; Motta et al., 2007; Tobin et al., 2020). In contrast, multiple studies have reported significant effects of CCs on soil health parameters following variable durations under of CCs (Blanco-Canqui and Jasa, 2019; Blanco-Canqui et al., 2015; Daryanto et al., 2018; Kim et al., 2020; Muhammad et al., 2021; Schipanski et al., 2014; Shackelford et al., 2019). The measured parameters were selected due to their strong correlation with SOM (Chahal and Van Eerd, 2019; Congreves et al., 2015; Fine et al., 2017; Hurisso et al., 2018). However, the short duration of the present study may have been inadequate to detect soil health benefits of the CCs, or the selected parameters were not adequately sensitive to the study conditions.

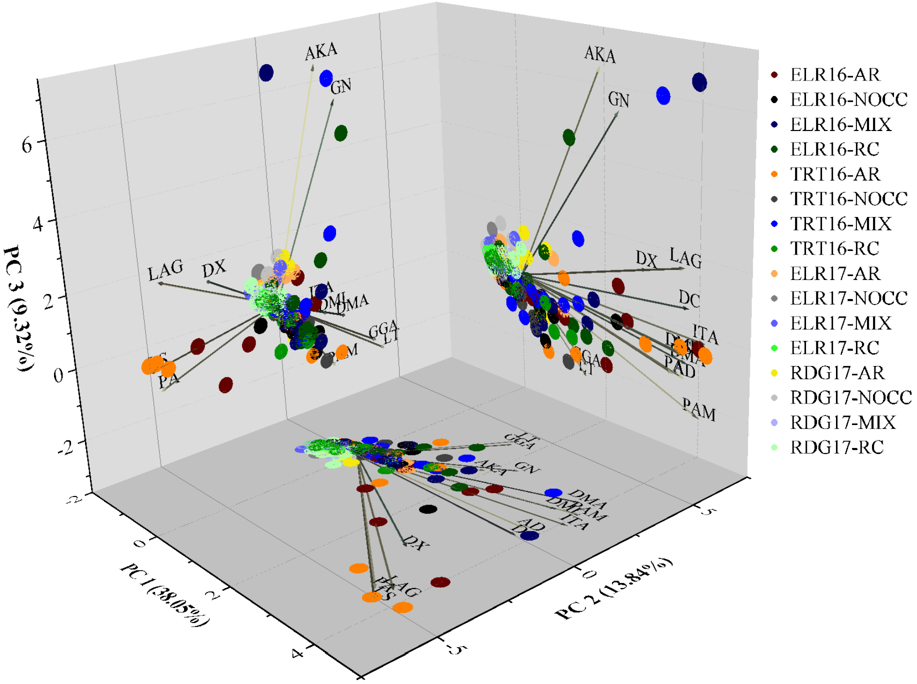


Figure 4.4. Two-dimensional projections of the biplot of the first three PCs of BIOLOG Ecoplate™ sole C utilization patterns as affected by site-year and interseeded cover crops. ELR = Elora, PTB = Peterborough, RDG = Ridgetown, AR = annual ryegrass, RC = red clover, MIX = AR + RC, and NOCC = no cover crop/control. C-source IDs are listed in Table 4.3.

It has been widely reported that the size of effects of CCs on soil parameters are dependent on biomass accumulation, years using CCs in rotation, the types of CCs and cash crops grown, and initial soil status (Blanco-Canqui and Jasa, 2019; Blanco-Canqui et al., 2015; Chen et al., 2019; Ladoni et al., 2016; McDaniel et al., 2014b; Wood and Bowman, 2021). Nevertheless, CC effects on labile SOM pools have also been found to be undetectable after longer periods than in our study, for e.g., water-extractable SOC after 3 years (Grebliunas et al., 2016) or POM-C measured from the 0 – 20 cm depth <6 years after CC incorporation (Duval et al., 2016) were also not reported to respond to CCs. On the other hand, Willson et al. (2001) reported increases in POM-N but not SMB-N in the first year following interseeding of AR, RC and their mixture into corn. In their meta-analysis of 302 studies in the USA, Nunes et al. (2020) concluded that the effects of management (tillage, cover crops, residue retention or perennial cropping) on soil health parameters were more detectable when implemented for at least three years.

The lack of responses of soil health parameters to CCs in the current study may also be due to the myriad of confounding factors such as variability in soil characteristics, temperature, and precipitation (Duval et al., 2016), corn stover biomass, native weeds, and other management practices at the four site-years which led to high variability in CC biomass. As soil health benefits of CCs are tied to CC establishment, high variability in CC biomass accumulation likely reduced the short-term effectiveness of these CCs. Moreover, corn stover and weed biomass also contributed to soil C inputs, and the contributions of CCs in one season may not have been adequate to trigger detectable responses of selected soil health parameters. In the fall, CC biomass was very sparse and amounted to only 3.9 % of total corn stover biomass. The volume of corn stover residues may dilute or confound the effects of the added CC biomass. Plots at PTB16 were cleared of all stover due to non-availability of harvesting equipment, hence only weed and CC biomass were returned to the soil. However, even at PTB16 where corn stover was removed from the plots, no CC effects were detected, suggesting that weed biomass (which accounted for 15 – 66% of total biomass added to the soil at this site) was sufficient to dilute any effects of cover crops. Weed biomass in NOCC was two-fold that in the CC treatments at PTB16, and this difference seemed to compensate for the absence of cover crops in the NOCC treatment. Research has shown that native weeds also promote microbial growth (Finney et al., 2017). Willson et al. (2001) observed smaller changes in SMB-N following the incorporation of RC than following the incorporation of a higher C/N ratio native weed which had higher C/N ratios.

In this study, composites of bulk soil samples were collected from the 0 – 15 cm depth between CC rows (as opposed to targeting rhizosphere soil or shallower depths (e.g., <10 cm). This which may have had a dilution effect on the observed responses due to the high spatial variability in microbial communities in bulk soils (Castle et al., 2019; Peacher and Meiners, 2020). Soil microbes are known to be differentially associated with free, aggregate-associated, or complexed SOM pools (Blaud et al., 2014) due to differences in the proportions, composition and reactivity of these pools (Poirier et al., 2005). Microbial activity also varies with aggregate size (Mendes et al., 1999), depth (Franzluebbers and Stuedemann, 2015), or proximity to plant roots (Dennis et al., 2010). Effects of cover crops on POM, SMB, or soil enzymes have been reported to be more pronounced in the top 2 cm (Wagner et al., 1995), 5 cm (Duval et al., 2016; Moore et al., 2014), 7.5 cm (Bremer et al., 1994) and 10 cm depth (García-González et al., 2018; Veras et al., 2016) but not at lower depths. However, using depth-weighted means and the maximum sampling depths as the controlling variable for evaluating responses in their meta analysis, McDaniel et al. (2014b) concluded that SMB was insensitive to cover crop effects when compared to corn monoculture in 39 studies with durations ranging 3 - 98 years.

Soil biological parameters varied greatly due to site-year effects. This is not surprising as there was inherent variability in environmental and management factors among the site-years (Table 3.2 and Supplementary Table 2). Temperature and precipitation variability often leads to differences in both the controlling (CC biomass or C and N contents) and response (soil health parameters) variables (Aerts, 1997; Kim et al., 2020). In this study, two site-years were excluded due to desiccation of all CCs at RDG in 2015 and non-emergence of RC at PTB in 2016. Among the successful site-years, there were also marked differences in operational protocols. For instance, cover crop planting in the first growing season was delayed at PTB, and N fertilizer rates and herbicide application rates at CC termination were greater at ELR than at PTB and RDG in both seasons (Table 3.3). More importantly, corn residues were removed from PTB16 and not at the other sites.

Higher POM-C, SMB-C, and SBR were observed at PTB16 than at ELR16 in the fall and spring (also POM-CN, BGA, and DHA), which suggest higher amounts of labile C may have been incorporated into SMB (Cotrufo et al., 2013) resulting in increased SBR (Mbuthia et al., 2015). Corn stover yield was not significantly different between ELR16 and PTB16 but cover crop and weed biomass at the time of fall soil sampling and in the spring were significantly greater at PTB16 than at ELR16, which suggests that cover crop and weed biomass at PTB16 had significant impacts on labile C and stimulated soil microbes. The removal of corn stover at PTB16 did not result in corresponding differences in the responses of soil health parameters compared to the other sites, further supporting the idea that inherent soil characteristics and residue quality, as opposed to quantity of added biomass (CCs/native weeds vs corn stover) were more important for the observed effects on soil health indicators in this study.

Greater POM-N at ELR16 may be due to more available N for weeds and CCs at ELR16, where 42.5 kg ha^{-1} more N were applied than that applied at PTB16. Greater POM-C at RDG17 than at ELR17 corresponded to the greater yield of corn stover with a higher C/N ratio at RDG17 than at ELR17. Differences in POM-C may be due to the different responses of fungi and bacteria to added crop residues (Kušlienė et al., 2014; Rousk and Bååth, 2007). On the other hand, higher POM-N, SMB-N, BGA, and DHA at ELR17 than RDG17 may be a result of higher levels of residual mineral N at the end of the fall at ELR17 than at RDG17, as well as significantly greater weed biomass at ELR17. The flush of CO_2 upon rewetting of air-dry soil was not significantly different between ELR16 and PTB16, or between ELR17 and RDG17. However, fCO_2 from sites in the 2016/17 season were significantly lower than those from the 2015/16 sites. The 2016/17 growing season was characterized by prolonged periods of drought, which can mask the effects of CCs and reduce soil microbial biomass (Vogel et al., 2013; Willson et al., 2001) or enzyme activities (Geisseler and Horwath, 2009) hence the soil microbial response to rewetting following air-drying was lower in the soil collected following a drought season. The fCO_2 values were not adjusted to account for differences in SMB, and this may have influenced the findings.

Analysis of CLPPs indicated across site-years, soil metabolic activities (denoted by AWCD) were significantly greater in AR soil than NOCC, RC, or MIX. PCA results showed no clear separation among CC treatments and may indicate lack of sensitivity of the BIOLOG CLPPs to short-term effects of CCs. BIOLOG EcoPlates have been used in a range of studies to assess microbial community responses to management changes (Bastida et al., 2008; Bucher and Lanyon, 2005; Drost et al., 2020; Rutgers et al., 2016). However, one limitation is that they only measure C substrate usage by a small proportion of aerobic bacteria. The current results suggest that despite this limitation of BIOLOG EcoPlates, the wide selection of C substrates may be useful for evaluating soil microbial responses to CCs in Ontario soils. According to the PCA, 45% of the C substrates available on the BIOLOG EcoPlates (14 substrates that were retained after applying the eigen-one criterion and eliminating redundant variables as described in Section 4.3.3) were able to explain 68% of the variation in metabolic profiles. This indicates potential for CLPPs to distinguish CC effects under conditions such as the ones in this study. The lack of clear distinctions among CC treatments or site-years (which were shown to be significant for most parameters in the global ANOVAs) indicates potential effects of management practices such as corn residue retention, tillage and fertilization (Adams et al., 2017; Mangalassery et al., 2015; Nivelle et al., 2016) or herbicide application (Escobar Ortega et al., 2021). These practices have the potential to drastically enhance or diminish soil microbial populations and activities, such that larger CC treatment effects are needed to produce significant shifts in metabolic profiles. Furthermore, soil microbial communities have inherent functional redundancies (Jia and Whalen, 2020; Louca et al., 2018), and shifts in community structure may not necessarily be reflected in the limited metabolic profiles measure through BIOLOG CLPPs.

The scores plot showed that AR and MIX treatments from PTB16 and ELR16 had the highest scores in PC1, PC2, and PC3. These site-years also exhibited greater AWCDs than ELR17 and RDG17. Nonlegumes such as AR produce more biomass relative to legumes, and have been reported to influence soil microbial communities and activities more than legumes (Blanco-Canqui and Jasa, 2019; Florence et al., 2019; Jian et al., 2020). The biomass and dynamics of bacterial and fungal communities are expected to change when plant residues are added to soil because different plant species have differential effects on soil microbial community structure (Bacq-Labreuil et al., 2019) or substrate use efficiency (Cotrufo et al., 2013). The CLPP show potential for increased soil metabolic activities with CC treatments that contained AR.

4.6. Conclusion

Soil health parameters measured in this study did not respond to CC inclusion. However, there was a significant trend ($p = 0.08$) which showed that POM-C increased in the order of NOCC<MIX<RC<AR. Also, AR significantly increased AWCD measured by BIOLOG CLPPs. These findings suggest that although most parameters were not significantly affected by CCs in the short term, there is potential for CCs to impact other parameters. Additional parameters with greater sensitivity, such as PLFAs, active C, or microbial functional diversity, need to be included in future research before bold conclusions or recommendations can be made. As soil health benefits of CCs are tied to CC establishment, high variability in CC biomass accumulation is a significant factor influencing the short-term effectiveness of these CCs for different objectives. Moreover, corn stover and weed biomass also contributed significant C inputs, adding to the complexity of the confounding effects on the soil health parameters under study. Future sampling protocols should also include both rhizosphere and bulk soils.

5. CARBON AND NITROGEN MINERALIZATION KINETICS DURING THE DECOMPOSITION OF CORN AND COVER CROP RESIDUES

5.1. Introduction

The maintenance of soil organic matter (SOM) is essential for sustainable crop production and good environmental stewardship. Crop residue retention following grain harvest presents considerable opportunities to build or maintain SOM (Lal, 2005; McDonald, 2010; Wilhelm et al., 2004). In corn-based rotations, high-yielding corn varieties with a relatively high harvest index of ~0.5 (ratio of harvested grain to total above-ground biomass at physiological maturity; (Fan et al., 2017)) provide large quantities of residues which can be retained in the field. However, managing corn residues can be challenging for producers, particularly when corn is followed by no-till crops such as soybeans (Aikins et al., 2019). Maintaining SOM without compromising subsequent crop yields is critical for economic and environmental sustainability in corn-soybean systems.

Large amounts of undecomposed corn residues retained on the soil surface can lead to delayed warming and drying of seedbeds, and can impede seeding operations (Vanhie et al., 2015a). Further, corn residue retention can increase the risk of temporary immobilization of added fertilizer-N during critical stages of seedling growth (Green and Blackmer, 1995; Taveira et al., 2020; Vanhie et al., 2015b). Over-winter (October – March) decomposition of corn residues amounts to 20 – 30 % biomass loss (Burgess et al., 2002; Stalker et al., 2015). Corn residues are comprised of distinct parts with variable quality metrics (e.g., C/N ratio, lignin, protein, or soluble sugars) (Pordesimo et al., 2005; Truong and Marschner, 2018). These portions generally decompose in the order: leaves > husks > stalks > cobs (Burgess et al., 2002; Tarkalson et al., 2008). Because stalks and cobs make up the greatest percentage of corn residue biomass and are the most resistant to decomposition, they present the greatest challenge for producers at the time of seeding subsequent crops.

In general, materials containing high water-soluble substances decompose the fastest (de Neergaard et al., 2002; Jensen et al., 2005); however the rate at which decomposition occurs depends on many factors, including quality (e.g., C/N and lignin/N ratios or proportions and speciations of polysaccharides), soil microbial activity, and environmental conditions (temperature, moisture, and soil physicochemical attributes) (Cabrera et al., 2005; Ghimire et al., 2017; Grzyb et al., 2020; Rosenzweig et al., 2017a; Schmatz et al., 2017). Adding diverse substrates into soils triggers diverse responses from the soil microbial community, including changes in C use efficiency (Lee and Schmidt, 2014). Including CCs in corn-soybean systems could shift microbial community structure or activity and may enhance the decomposition of corn residues in the spring, thereby reducing the risks associated with corn residue retention (Quemada and Cabrera, 1995).

Improved plant residue diversity with CC inclusion enhances the diversity of substrates available for soil microbes compared to residues available in monoculture cropping systems (Cong et al., 2015; Schimel and Schaeffer, 2015). Also, CC residues have the potential to enhance residue decomposition rates by increasing substrate quality and soil microbial activity (Chahal and Van Eerd, 2020; McDaniel et al., 2014a). Cover crop residues with lower C/N ratios and lignin contents are expected to improve corn residue decomposition rates and reduce N immobilization (Cleveland et al., 2014; DuPont et al., 2009; Whitehead et al., 1979). These potential benefits of CC inclusion may be of consequence to crop residue management in conventional annual cropping systems such as the predominant corn-soybean systems in Southern Ontario.

The addition of crop residues can also lead to the stimulation of native soil C decomposition (priming) (Thiessen et al., 2013; Wang et al., 2015). In an assessment of 139 cover-cropped plots, Poeplau and Don (2015) reported increases in SOC from CC introduction in 91% of the cited studies. The authors attributed the few instances in which decreases in SOC were observed to potential priming of native SOC by the CCs. Residue C/N ratios influence the magnitude and direction of priming by altering the composition and structure of soil microbial communities (Cabrera et al., 2005; Liang et al., 2017). Lange et al. (2015) reported that the sequestration of new C from added residues and the release of old C through respiration of native SOM are highly correlated. It is not known if residues from interseeded AR modify the decomposition of corn residues, or what the implications of CC and corn residues mixtures are for SOM decomposition.

A laboratory incubation study was set up to evaluate effects of RC and AR residue amendment to soil on C and N mineralization (Cmin and Nmin, respectively) during corn stover decomposition. The hypothesis was that the lower C/N ratio (<20) and lignin contents of interseeded RC and AR residues (at termination in early spring) can increase the rate of corn stover residue decomposition, reduce N immobilization (Aulakh et al., 1991; Kaboneka et al., 1997; Odhiambo and Bomke, 2000) and consequently reduce the risk of temporary soil N deficiency during the early season following corn crop (Ehaliotis et al., 1998; Green and Blackmer, 1995). Understanding the implications of CC residues on corn stover decomposition is important for informing management decisions such as the CC species, and termination method and timing.

5.2. Materials and Methods

5.2.1. Soil and Residues

Bulk soil samples were collected from the 0 - 15 cm depth using augers (to mark depth) and scoops from the RDG16 and PTB16 experimental sites on 17 and 18 June 2017, respectively. Soil sampling sites had been seeded to corn in 2016. Stones and visible plant debris were manually removed, and the soil was passed through a <2 mm sieve and thoroughly mixed before air drying in the laboratory under running fans for a week before the start of the incubation. Prior to treatment addition, a subsample was collected from the air-dry soil for characterization of soil properties. Mineral N was extracted using 2M KCl and determined by flow injection analysis (Maynard and Kalra, 2008) using a continuous flow analyzer (Bran Luebbe AA3, Seal Analytical Inc., Mequon, WS). Total C and N were determined by dry combustion using a CNS analyzer following carbonate removal with H_2SO_3 (Skjemstad and Baldock, 2008)]. Available P (Olsen P) was measured using the ascorbic acid-molybdate method (Olsen et al., 1954; Murphy and Riley, 1962]. Exchangeable Ca, Mg, and K, were measured by atomic absorption spectroscopy following extraction of a 1:4 (w/v) soil:1M NH_4OAc suspension at pH 7 (Hendershot et al., 2008c)]. pH and electrical conductivity were determined using a 1:2 soil/water suspension (w/w) (Sikora, 2014)]. The sandy loam soil had lower OM, pH, Ca, and CEC than the silt loam. On the other hand, mineral N (NO_3^- + NH_4^+), K, Mg, and Na, were greater in the sandy loam

Corn stover and aboveground CC residues were collected from all sites prior to CC termination in early May 2016 and 2017. The residues were dried at 60°C for ~72 h prior to grinding and passing through sieves to retain the 2 – 5 mm fraction. Total C and N was determined by dry combustion on a vario MAX cube CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The lignin content of the residues was determined using H_2SO_4 digestion (Klason method) as described by Kirk and Obst (1988). Residue characteristics are presented in Table 5.1.

5.2.2. Experimental Design and Treatment Setup

The experiment was a completely randomized 2 × 6, full-factorial combination of two soils (a sandy loam from RDG16 and a silt loam from PTB16) and six residue treatments: (i) corn (CN); (ii) annual ryegrass (AR); (iii) red clover (RC); (iv) CN + AR; (v) CN + RC; and (vi) a no residue control (NOCC). The corn residue incorporation rate was 7.4 mg DM g^{-1} soil, while AR and RC were applied at 1.80 and 1.98 mg DM g^{-1}, respectively. The CN residue rate corresponded to an optimal grain yield of 12 Mg ha^{-1} with a harvest index of 0.5 (based on data from RDG15, which had the highest grain yield for the study period). Cover crop residue rates were based on the amount required to provide 0.7 mg C g^{-1} soil, which is three times greater than the average C loading rate observed for CCs in the 2015/16 season (0.21 mg C g^{-1} soil). Overall, the N added in CN residues (90 mg kg^{-1} soil) was at least 100 and 30% more than from AR (45 mg kg^{-1} soil) or RC (69 mg kg^{-1} soil), respectively.

Table 5.1. Selected properties of soils and residues used in the incubation study.

Parameter	Soil		Crop Residue		
	Sandy loam (Ridgetown)	**Silt loam (Peterborough)**	**Corn**	**Annual ryegrass**	**Red clover**
pH	7.1	7.6			
Total N, g kg^{-1}	1.58	2.51	6.0	23.2	33.4
Inorganic N (NO_3^- + NH_4^+), mg kg^{-1}	41.6	26.3	-	-	-
Total organic N, g kg^{-1}	1.54	2.48	-	-	-
Total C, g kg^{-1}	20.2	33.1	-	-	-
Total organic C, g kg$^{-1\dagger}$	16.3	22.1	420	354	388
C/N ratio	10.3	8.8	70.3	15.3	11.6
Lignin, g kg^{-1}	-	-	57.2	27.4	104
Lignin/N ratio	-	-	9.53	1.18	3.1
Cation exchange capacity, meq 100g^{-1}	10.3	26.4	-	-	-
Clay, %	2.54	3.95	-	-	-
Silt, %	47.8	60.5	-	-	-
Sand, %	49.6	35.5	-	-	-

Prior to residue addition, bulk soils were sieved (< 2mm) and thoroughly homogenized. For each treatment, 200g soil were weighed into 500-mL Mason jars. All jars were moistened to 40 ±5% WFPS and preincubated for three days at 24 ± 1°C in the dark. Following preincubation and prior to treatment addition, three replicates of each soil were analyzed to determine the background levels of SMN and SMB. Eighteen replicates of the 12 treatments were prepared to facilitate destructive sampling of three replicates at six time points (total = 222 experimental units).

Carbon mineralization

Cmin was determined following a modification of the procedure described by Hopkins (2008). In each of 36 1-L mason jars (12 treatments × 3 reps), 200 g of soil (dry wt.) were thoroughly mixed with their respective residues of corn and/or CCs. The jars were tapped to pack soils to bulk densities approximately 1 g cm^{-3}, and RO water was added to adjust moisture content to 60 % WFPS. Initial weights of each jar were recorded and used as benchmarks to maintain constant moisture content throughout the incubation period by weighing once every 3 - 7 d and watering as needed. A 100-mL cup containing 50 mL of 1 M NaOH was placed in each jar before tightly sealing with a metal cap. The jars were incubated in the dark at 24 ± 1°C. Throughout the incubation, non-destructive sampling was conducted at 2, 4, 7, 10, 14, 21, 28, 38, 48, 58, 73, 88, and 102 d. The concentration of the NaOH in the alkali traps was reduced to 0.5 M after sampling on Day 10, as titrations indicated this lower concentration would be adequate to trap the CO_2 evolved in this study. Evolved CO_2 was analyzed by back-titration of the NaOH with standardized 1 M HCl. At each sampling time, jars were left open for 30 – 40 min for aeration before placing fresh traps and recapping. Net Cmin was calculated as the difference between the CO_2-C evolved from the residue-amended soils and the unamended soil.

Nitrogen Mineralization

Following the 3-d preincubation period, 200 g of soil (dry wt.) were prepared as described above for Cmin. The jars were left uncapped to allow aeration and incubated in the dark at 24±1°C throughout the incubation period. Destructive sampling was done at 0, 3, 7, 14, 30, 60, and 98 d after treatment application. At each sampling time, the appropriate jars were removed from the incubator for the analyses of SMN (NO_3^- + NH_4^+). SMN was determined by extraction of 5 g sieved (<2mm) field-moist soil with 50 mL 2 M KCl followed by flow injection analysis (Maynard and Kalra, 2008) using a continuous flow analyzer (Bran Luebbe AA3, Seal Analytical Inc., Mequon, WS).

5.2.3. Statistical Analyses

Net Cmin and Nmin were calculated as the differences between cumulative CO_2 and SMN levels, respectively, in the unamended control and those in the amended treatments at each sampling time. Soil and residue effects on net cumulative Cmin and Nmin were subjected to two-way ANOVAs using Proc GLIMMIX in SAS 9.4 (SAS Institute Inc., 2019). Soil, residue, and the soil × residue interaction were fixed effects in the models, while sampling time was the repeated variable (for Cmin and Nmin) and replicates were the random effect. Model assumptions of homogeneous variance and normality of residuals were tested using the Shapiro-Wilk's test.

Additionally, data for Cmin was fit to first-order exponential models to determine the mineralization rates and the potentially mineralizable organic C pool (C_0 in Equation 6). The evolved CO_2 measured at 14 -98 d were used for curve fitting because raw data from the unamended control indicated that mobilization due to the initial flush upon rewetting was still happening at sampling times <14 d (Benbi and Richter, 2002; Sharifi et al., 2008). Model evaluation and curve-fitting were done using PROC NLIN in SAS v9.4 (SAS Institute Inc., 2019) and OriginPro 2021 (OriginLab Corporation, 2021), respectively. The fitted models were:

$$\mathrm{C_{min}} = \mathrm{C_0}(1 - \exp^{(-k_C \mathrm{t})}) \qquad [2]$$

where Cmin is the cumulative evolved C; C_0 is the potentially mineralizable C (PMC); k_C is the first-order mineralization rate constant for Cmin; and t is the incubation time.

All model parameters were analyzed by ANOVA ($\alpha < 0.05$) using Proc GLIMMIX in SAS v9.4 (SAS Institute Inc., 2019). Soil and residue effects were fixed effects, and the replicates were the random effects in the model.

5.3. Results

5.3.1. Net Cumulative Carbon Mineralization

There was a significant soil × residue × day interaction on net cumulative Cmin (Table 5.2). In the silt loam, no significant differences were observed between AR and RC or among the corn-amended treatments, CN, CN+AR, and CN+RC, for the duration of the incubation. From 7 to 102 d, net Cmin from AR and RC was significantly lower than CN, CN+AR, and CN+RC in both soils. At these later stages of decomposition, all three corn-amended treatments (CN, CN+AR, and CN+RC) had significantly greater net Cmin than the CC-only treatments (Figure 5.1), a trend which persisted in both soils over the duration of the incubation period. From Day 48 to the end of the incubation period, net cumulative Cmin was similar between AR and RC treatments in the silt loam but was significantly lower in AR than in RC in the sandy loam. At the end of the incubation (Day 102), net Cmin from corn-containing treatments did not significantly vary between the sandy loam and silt loam. Net cumulative Cmin from RC did not vary significantly between the sandy loam and silt loam (374 vs 457 mg CO_2-C kg^{-1}, respectively), whereas that from AR was significantly greater in the silt loam (442 mg CO_2-C kg^{-1}) than the sandy loam (208 mg CO_2-C kg^{-1}).

Table 5.2. Net C and N mineralization in soil amended with corn and cover crop residues.

Effect	**Net Cmin**[†]	**Net Nmin**
	$mg\ kg^{-1}$	
Soil (S)		
Sandy Loam	446	-20.1
Silt Loam	495	-16.2
Residue (R)		
Control	-	-
Annual ryegrass	280	-1.2
Red clover	315	-7.8
Corn	592	-30.1
Corn + Annual ryegrass	651	-25.6
Corn + Red clover	672	-26.1
Day (D)[‡]		
2 (*3*)	126	-21.2
4	245	-
7	330	-16.9
10	409	-
14	464	-18.0
21	508	-
28 (*30*)	552	-34.5
38	591	-
48	649	-
58 (*60*)	682	-17.4
73	710	-
88	738	-
102 (*98*)	734	-1.0
	p-value[§]	
S × R × D	0.0002	< 0.0001

[†] Net Cmin or Nmin = evolved CO_2-C or mineral N (NH_4^+ + NO_3^-) in residue-treated soil minus that in unamended soil (control) at Day = *t*; SMB-N and SMB-C, soil microbial biomass N and C, respectively; SMBC-CN, soil microbial C/N ratio; CUE, microbial C use efficiency.

[‡] Incubation days in italics are when destructive sampling for the N mineralization assay was done.

[§] Soil and residue main effects were significant at $p<0.001$

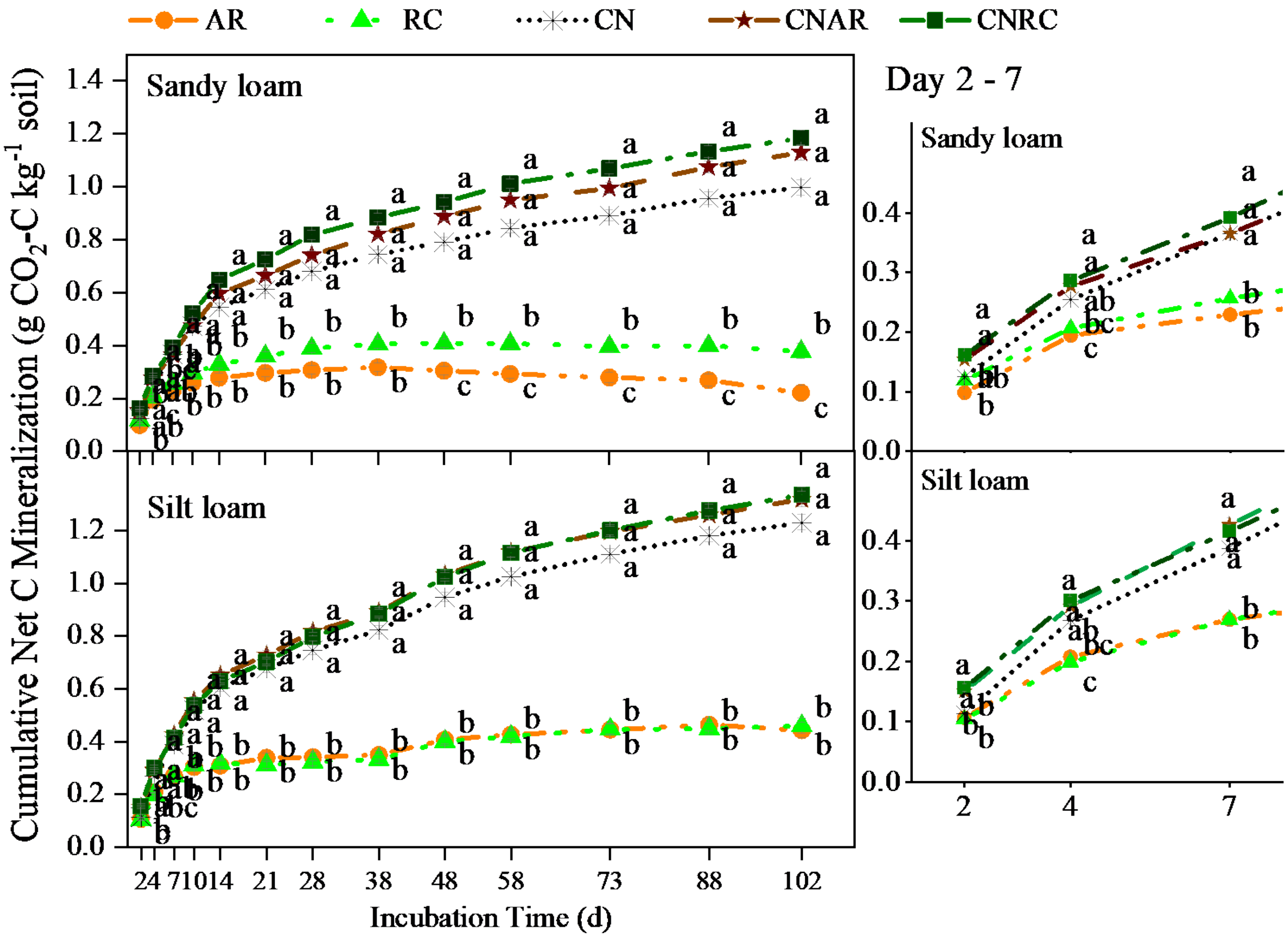


Figure 5.1. Cumulative net C mineralization in soils amended with corn and cover crop residues. Days with the same letters are not significantly different according to the Tukey-Kramer mean separation procedure (P<0.05).

5.3.2. Carbon Mineralization Kinetics

Potentially mineralizable C (parameter C_0 in Equation 6; mg C kg^{-1} soil; Table 5.3) was significantly greater in corn-amended treatments than those without corn in both soils. In the sand, C_0 was significantly greater in CN+RC than in CN. In the silt loam, C_0 was not significantly different among corn residue-amended treatments (CN+AR, CN+RC, and CN), or those without corn (CTRL, AR, and RC). The predicted Cmin rate constant (parameter k_C in Equation 6) increased with residue addition in both soils (Table 5.3). Residues increased k_C by 152 – 232% and 250 – 445% in the sandy loam and silt loam, respectively (Figure 5.3). The k_C for AR and RC was significantly greater in the sandy loam than in the silt loam, but there were no differences between soils for CTRL, CN, CN+AR, and CN+RC.

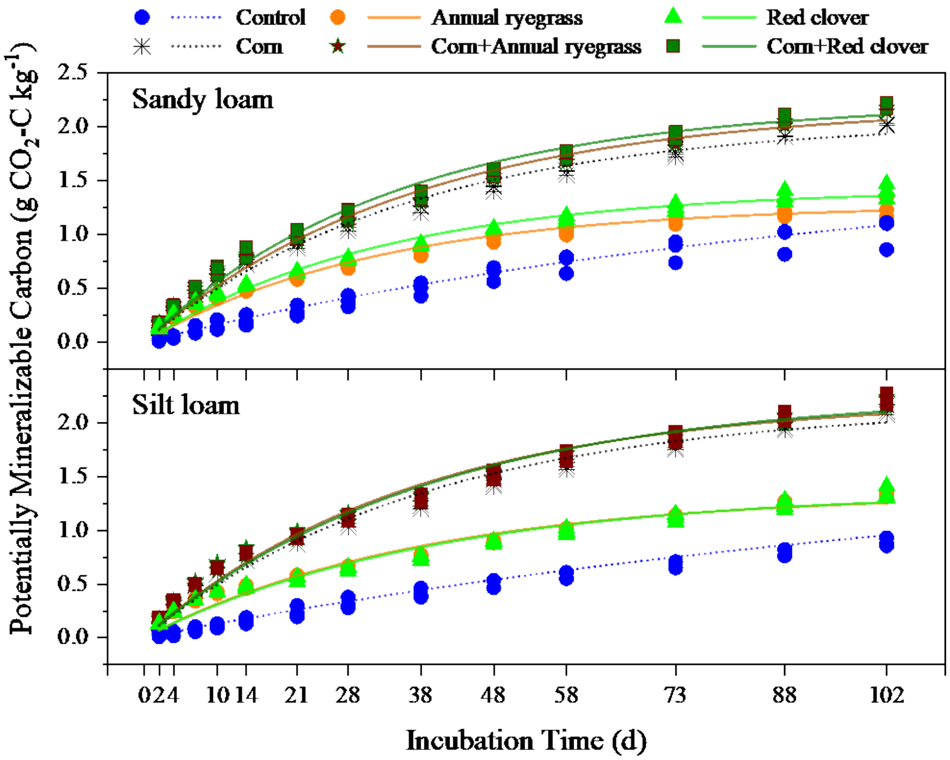


Figure 5.2. First-order exponential model fits for C mineralization data for sandy loam and silt loam soils amended with corn and cover crop residues

Table 5.3. First-order kinetic model parameter estimates for C and N mineralization dynamics in soil amended with corn and/or cover crop residues.

Treatment	**C_0**[†]	**k_C**
	g kg^{-1}	
Soil (S)		
Sandy loam	1.73	0.026 a
Silt loam	1.76	0.023 b
Standard error	*0.02*	*0.001*
Residue (R)		
No residue	0.97 d	0.021 b
Annual ryegrass	1.35 c	0.026 a
Red clover	1.44 c	0.025 a
Corn	2.15 b	0.025 ab
Corn + Annual ryegrass	2.26 ab	0.025 a
Corn + Red clover	2.30 a	0.025 a
Standard error	*0.035*	*0.001*
	p-value	
S	0.363	< 0.001
R	< 0.001	0.01
S × R	0.101	0.387

[†] C_0 is the potentially mineralizable C and k_C is the first-order rate constants for C mineralization.

5.3.3. Net Nitrogen Mineralization

There was a significant interaction of soil, residue type, and incubation time on net Nmin (Table 5.2). Overall, net Nmin was not significantly different between CC-only treatments (AR and RC), or among CN-containing treatments (CN, CN+AR, and CN+RC) throughout the incubation period in both soils (Figure 5.2). On the other hand, in the sandy loam, net mineralized N contents from the corn-containing treatments were significantly lower than those from AR at 3 – 30 d, or from RC at 14 – 30 d. No treatment differences were observed from Day 60 to Day 98. In the silt loam, AR was significantly greater than CN from 14 to 30 d, and CN+AR and CN+RC from 14 to 60 d. On the other hand, Nmin in RC-amended soils was not significantly different from all treatments at all sampling times, except from CN+AR at 30d. The highest N immobilization was in the corn-containing treatments at 30 d in both soils, ranging 50 - 60 mg N kg^{-1}. By the end of the 98-d incubation period, N immobilization did not significantly differ between the sandy loam and silt loam for any residue treatments: AR, -0.71 vs -8.18; RC, -1.47 vs -0.97; CN, -2.33 vs 0.49; CN+AR, 1.47 vs 0.20; and CN+RC, -0.86 vs 2.86 mg N kg^{-1}; respectively. In the sandy loam, immobilization had been significantly reduced in the AR and RC treatments at 7- and 14-d of incubation, respectively (Figure 5.2). On the other hand, N immobilization lasted for 60 d in the CN+AR treatment, and 98 d in CN and CN+RC. In the silt loam, immobilization lasted for the whole duration of the incubation for all treatments.

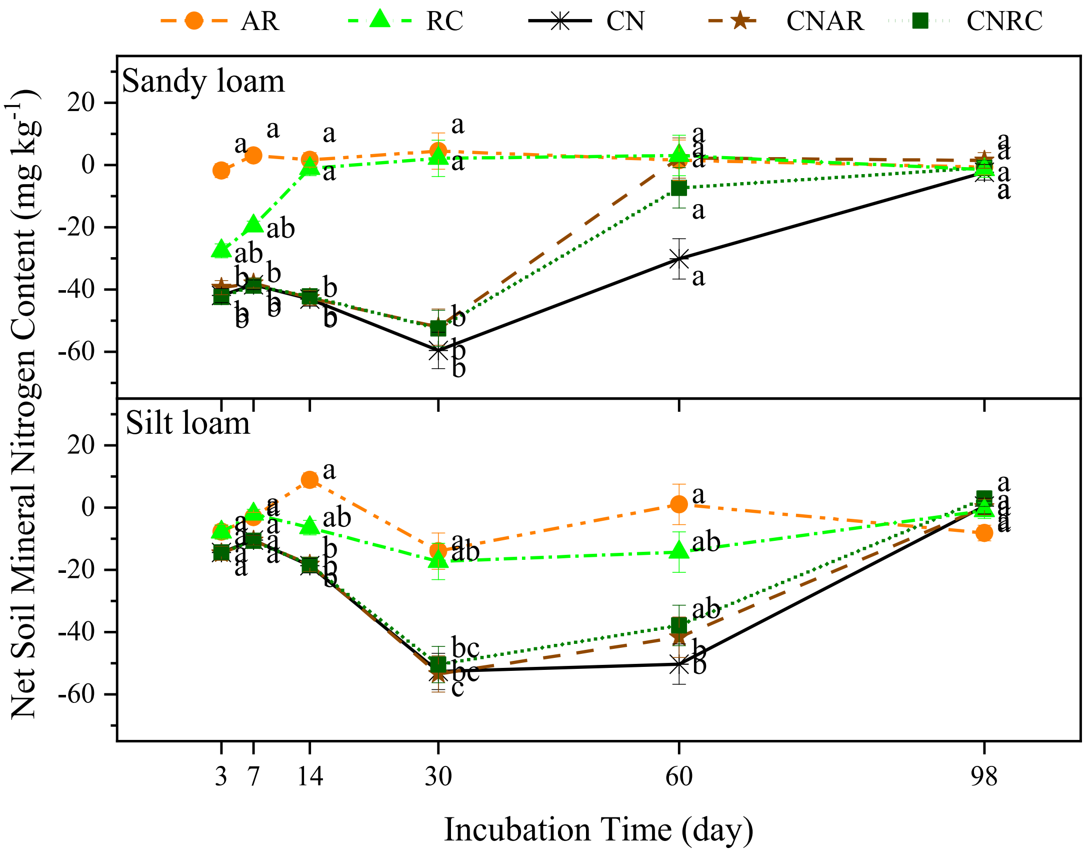


Figure 5.3. Net mineral N content (NO_3^--N+ NH_4^+-N) during the decomposition of corn and CC residues

5.4. Discussion

The two soils used in this incubation study varied not only in clay content (texture), but also in the amounts of SOC and N (Table 5.1). The silt loam had greater SOC and SON and CEC. On the other hand, the sandy loam had more mineral N (NO_3^- + NH_4^+). Corn stover residue greatly varied from AR and RC in having a higher C/N and Lignin/N ratios, both of which are fundamentally important in influencing residue turnover in soil (Stewart et al., 2015; Thevenot et al., 2010). The mineralization potentials of C estimated from first-order kinetic models is highly correlated with residue mass loss (Parshotam et al., 2000; Reis et al., 2011; Wienhold et al., 2011; Yanni et al., 2010), hence parameter estimates from a first-order kinetic model were used in the study to estimate the extents and rates of CN and CC residue decomposition.

Throughout the incubation period, net cumulative Cmin observed for treatments containing CN residues were at least triple those for the CC-only treatments. This was consistent with the greater amount of C added in CN residues, which led to greater soil respiration compared to the relatively smaller quantities in AR and RC (Duval et al., 2016; Eberwein et al., 2015). Due to their meager contributions, the addition of CC residues to CN in CN+AR and CN+RC treatments did not enhance net cumulative Cmin relative to CN alone. The kinetic model showed that C_0 in CN + AR was only slightly increased relative to CN, while that of CN + RC was significantly increased compared to CN.

When different residues are mixed, they can have neutral (additive), positive, or negative (non-additive) effects on each other’s decomposition kinetics (Gartner and Cardon, 2004; Li et al., 2013; McTiernan et al., 1997). Non-additivity has also been reported for different parts of the same crop, e.g., wheat (*Triticum aestivum L.*), rye (*Secale* *cereale L.*), oat (*Avena saliva L.*), and crimson clover (*Trifolium incarnatum L.*) in Quemada and Cabrera (1995). Overall, the combination CN with AR or RC did not result in non-additive effects, as shown by the Cmin or C_0 values for the mixtures compared to the single residues. Non-additivity is caused by the interactions of different residues on decomposer community structure (abundance and activity) and variable patterns of mass loss or nutrient release from the decomposing residues (Gartner and Cardon, 2004).

Although small amounts of C were supplied from both AR and RC, net cumulative Cmin from AR in the silt loam was double that in the sandy loam. Additionally, the Cmin rate was greater in the silt loam than in the sandy loam when CC residues were added. This suggests that AR and RC may have triggered greater responses of the microbial community in the silt loam than in the sandy loam due to the higher native SOC levels in the silt loam (Gillis and Price, 2016; Xu et al., 2016). Several studies have showed that the addition of fresh crop residues can enhance the decomposition of native soil OM (Fontaine et al., 2004; Qiu et al., 2016; Rosenzweig et al., 2017b; Schmatz et al., 2017; Stewart et al., 2015; Wang et al., 2015). The differences between soils were not observed when CN residues were added, and it appears that residue influences on native SOC are more evident under C-limiting conditions.

Net cumulative Cmin did not differ between AR and RC in the silt loam, but significantly more C was evolved from AR than from RC in the sandy loam. This greater response in the sandy loam may be due to the lower native SOC content than the silt loam. Red clover had a lower C/N ratio than annual ryegrass, which may lead to faster decomposition (Cleveland et al., 2014). (Sievers and Cook, 2018; Singh et al., 2020). However, due to a much higher lignin content, which slows down decomposition rates

(Stewart et al., 2015; Yanni et al., 2010), the decomposition rate from RC was similar to AR. We did not characterize the lignin pools in these residues, but these results suggest that the lignin pools in RC were not labile (Thevenot et al., 2010). First-order kinetic model results showed that combining CN residues with CCs did not significantly enhance the amount of potentially mineralizable C ($C_{0.}$) relative to adding CN residues alone.

Residue quality is important in controlling Cmin kinetics as it determines the types of substrates available for soil microbes (Chen et al., 2014; Johnson et al., 2007; Magid et al., 2004). In both soils, the predicted Cmin rates (k_C) of all residue-amended soils were higher relative to unamended soil. The comparable k_C from AR and RC, despite the meager amounts of C that were added in CC residues compared to CN, suggest that the C from CC residues may have been predominantly easily-decomposable C forms, such as water-soluble polysaccharides (de Neergaard et al., 2002; Jensen et al., 2005). Also, as the quantity and quality of labile C is known to influence Cmin rate (Liang et al., 2017; Truong and Marschner, 2018), the C forms from CN residues may not have been as easily-decomposable as those from AR and RC. The k_C values predicted in this study suggest that the Cmin rate in the CTRL was influenced by stable C whereas those in amended soils were influenced by labile C (Trinsoutrot et al., 2000).

Overall, the C_0 was similar between the two soils regardless of type of residue added, or differences in native SOC content (36% higher SOC content in the silt loam than the sandy loam at the beginning of the incubation). These predicted C_0 values were very close to the measured cumulative Cmin values after 102 d. Measured Cmin and C_0 predicted from the model showed that 6 - 11% of the total SOC (native + residue C) was mineralized in each treatment, suggesting that the native SOC may have been mostly recalcitrant

(Trinsoutrot et al., 2000), or that most of the residue C supplied at the higher C rate in CN-amended treatments was not easily-decomposable.

Greater k_C values were predicted for the sandy loam soil relative to the silt loam. High clay and SOM contents are associated with smaller proportions of macropores, hence reduced aeration in the silt loam compared to the sandy loam. Coarse soils have more aeration and allow for faster diffusion of extracellular enzymes which are important for rapid decomposition of labile C pools (Müller and Höper, 2004; Xu et al., 2016). The relationship between soil texture and enzyme diffusivity has also been suggested as a mechanism by which decomposition rates are generally faster in coarse soils or soils with more macro-aggregates (>2mm) (Frøseth and Bleken, 2015). Although microaggregates (found in soils with higher OM and clay contents) support greater microbial diversity (Bach et al., 2018) and soil aggregation (Bimüller et al., 2016), access of soil microbes to organic substrates can be limited in soils with higher clay contents.

No additional N was added to all treatments in this study, to imitate decomposition that starts immediately following CC desiccation (prior to seeding and fertilization of the preceding crop). Because SMN levels were low, net immobilization was observed from all treatments, albeit for variable extents and durations. At its peak, N immobilization was greater in CN-containing treatments compared to AR and RC. This was expected as more residues with higher C/N ratios were applied in the CN-containing treatments (Kaboneka et al., 1997). Overall, net N immobilization lasted longer (102 d) in the silt loam than in the sandy loam (60 d). Most immobilized N from AR, RC, and CN+AR had been recovered before the end of the 98-d incubation period in the sandy loam. The differences in the durations of net immobilization highlight the differences in clay and SOC contents between the two soils.

5.5. Conclusions

Overall, Cmin and Nmin rates were greater in the sandy loam than the silt loam, highlighting the influence of the higher clay content and potential SOM protection in the silt loam or the greater aeration potential of the sandy loam. The addition of CN and CC residues enhanced Cmin and Nmin in both soils. Due to differences in the amount of residue C applied, Cmin from CN-amended treatments was at least three times greater than from CC-only treatments. The combination of CCs and CN in the CN+AR and CN+RC treatments did not lead to significant differences in net Cmin and Nmin. However, results of the first-order kinetic model indicate increases in predicted PMC and PMN in both soils and in the silt loam, respectively. Despite the lower application rates of CC residues, Cmin rate constants of AR and RC were comparable to those of CN-amended treatments, indicating the presence of more easily-decomposable C in the CC residues. The addition of CN residues led to greater N immobilization than CC residues alone. In the sandy loam, residue mixtures (CN+AR and CN+RC) had shorter net immobilization periods than CN only. This suggests potential for faster N re-mineralization during the growing season, which could improve crop N supply. These results show that CC residues may have a positive but minimal impact on CN residue decomposition.

6. GENERAL DISCUSSION

Cover crops (CCs) provide an opportunity to maintain soil cover and introduce diversity in Canadian cropping systems. In Chapter 2, the potential relevance of CCs as a conservation strategy in Canadian agricultural landscapes was discussed. Information from Statistics Canada (2017b) and AAFC (2020), which was based on the 2016 Census of Agriculture, was analyzed to identify opportunities for CC inclusion in the diverse Canadian cropping systems. Available data shows that crop rotations have been widely adopted and bare fallows are almost extinct across Canada. However, trends in recent years show significant shifts towards highly simplified rotation systems with lower crop diversity (Liu et al., 2020; McRae et al., 2000). The predominant cropping systems in the different Canadian systems vary significantly among Eastern, Western and Atlantic Provinces, but there is limited crop diversity within each region.

Consistent with research across the globe (Florence et al., 2019; Kim et al., 2020; Ruis et al., 2019), existing published research from across Canada shows high variability in CC establishment and effectiveness for enhancing crop yields, N supply, weed suppression, and soil health indicators. This variability is due to several key factors, including climate, management, cash crops, and CC species. Based on current knowledge from domestic and other North American studies (Blanco-Canqui et al., 2015; Kim et al., 2020; Poeplau and Don, 2015), it is apparent that CC research needs to be diversified within the context of each region to customize adoption recommendations for diverse cash crop-CC combinations. Existing research also suggests that CC adoption could fast-track conservation targets within the diverse agricultural regions of Canada.

This PhD thesis research was based on the need to evaluate the potential for CC inclusion through interseeding between standing in Ontario. Two CC species, AR and RC, and their mixture (MIX) were interseeded into corn at three sites in southwestern and central Ontario in 2015-2017. Results showed variable CC biomass, and C and N accumulation, which were highly influenced by the site-year and CC species. Due to negligible or failed CC establishment, two site-years of data (RDG15 and PTB16) were dropped from the assessment. AR seemed more resilient to drought and extreme temperatures while RC failed completely at two site-years which experienced above average temperatures and drought. According to correlation analysis, corn grain yield, grain and stover C, and corn N uptake were negatively correlated with accumulated CC biomass, C, and N. Based on regression analysis, corn grain and stover C accumulation explained significant variation in CC biomass in the fall whereas fall CC and weed biomass explained 74% of the variation in spring CC biomass (reshoots). These relationships suggest the influence of corn on the accumulation of interseeded CCs biomass in the fall. When interseeding is done at the V4-V6 stage, corn would have reached a very competitive stage and rapidly accumulating biomass. Corn canopy growth and weeds were a significant factor in limiting the rate of CC growth in the summer-fall phase of the CC cycles. At RDG15, the corn variety rapidly accumulated biomass and evidently limited CC growth, as all established CCs totally failed mid-season. The selection of CC species therefore needs to focus on fast-growing varieties which can thrive under reduced light intensity.

Corn grain yield or stover biomass were within the ranges reported for these regions (Janovicek et al., 2021; Stoeckli et al., 2021), and were not reduced by the interseeded CCs. The significant variations in corn yield among site-years were disproportionately influenced by mid-season drought and extreme temperatures. Also, regression analyses showed that fall weed biomass explained significant portions of the variation in corn and CC biomass yields. Of the three sites in this study, PTB was the least intensively managed site compared to the highly efficient management (e.g., seedbed preparation and weed control) at the more established ELR and RDG sites. At PTB, fall and spring weed biomass yields were significantly greater than at ELR or RDG, and this translated into lower corn yield. However, at this site, the effectiveness of CCs in suppressing weeds was also more pronounced. This suggests a potential for CC use for weed suppression in corn systems with reduced/minimal herbicide use.

Potential risks of yield reduction and competition for water and nutrients are some of the reasons for poor CC adoption (Tonitto et al., 2006), hence the second research question of this thesis was whether interseeded CCs would present significant competition for N with corn in Ontario. Corn N uptake, tissue N concentrations, and residual SMN at harvest were measured. CCs did not significantly reduce the amount of N taken up by corn or that remaining in the soil at the end of the two seasons. Stepwise regression showed that stover N could predict SMNf while grain C and N could predict SMNs. These results also show that the level of CC establishment in this study may not be sufficient to produce detectable effects on soil N supply. However, instances where significant CC biomass accumulation were not accompanied by corresponding increases in agronomic benefits have been reported (Marcillo and Miguez, 2017; Miguez and Bollero, 2005; Tonitto et al., 2006).

It has been noted that part of the hesitation of producers towards CC adoption is due to the difficulty in quantifying benefits, especially those that could directly offset the potential risks (Delgado and Gantzer, 2015) and additional costs associated with the inclusion of CCs to already financially burdened producers (Dunn et al., 2016; Roesch-McNally et al., 2018b). In Chapter 3, dynamic soil health indicators were measured to assess short-term effects of interseeded CCs. Except for microbial community level physiological profiles using BIOLOG EcoPlates ™, none of the measured indicators were significantly affected by CCs. As the effectiveness of CCs ultimately relied on the quantities of biomass added to the soil, the results obtained in this study also highlighted the limited sensitivity of selected indicators in the short-term duration of this study. Based on the BIOLOG data, AR showed significant potential to increase soil microbial diversity. Overall, this study confirms the need for more research to identify suitable soil health indicators for short-term assessment of management impacts.

To provide additional insight on the potential mechanisms of CC impacts on soil health and sustainability in corn cropping systems, the dynamics of corn stover decomposition in the presence of AR and RC residues were evaluated. As expected, the addition of CN and CC residues enhanced net cumulative Cmin compared to the unamended soil. However, due to the vast differences in the amounts and quality of C applied in CN and CC residues, Cmin from CN-amended treatments was at least three times greater than from CC-only treatments. More importantly, mixing CCs and CN (i.e., CN+AR and CN+RC treatments) did not lead to significant differences in net Cmin and Nmin. Results of the first-order kinetic model indicated increases in predicted PMC in both soils and PMN in the silt loam. Despite the lower application rates of CC residues, Cmin rate constants of AR and RC were comparable to those of CN-amended treatments, indicating the presence of more easily-decomposable C in the CC residues. Overall, the addition of CN residues led to greater N immobilization than CC residues alone. In the sandy loam, CN+AR and CN+RC had shorter net immobilization periods than CN alone. This suggests potential for faster re-mineralization of N during the growing season, which could improve crop N supply. These results show that CC residues may have a positive but minimal impact on CN residue decomposition.

From both the field and controlled incubation experiments, the notable conclusion is the complexity of the systems in which CC benefits are evaluated. It is difficult to make firm conclusions about the effectiveness (or lack thereof) of CCs when all confounding factors are considered. Using stepwise regression and accounting for weed and main crop biomass highlighted the importance of successful CC establishment, if significant benefits are to be observed. Although the effects of CCs on soil health were not significant under the conditions of this study, the results highlighted potential for weed suppression, and negligible yield or N penalties for grain corn when CCs are interseeded into standing corn. The present study was one of the few studies focusing on interseeding CCs into grain corn, and only two species were assessed. It is important to remember that there are many CC species with unique capacities for establishment under different environments and management systems. As the current study was in its infancy, caution must be used when interpreting the findings.

6.1. RECOMMENDATIONS FOR FUTURE RESEARCH

No single study can provide all answers required for policy-making or technical recommendations. As noted by Van Eerd (2018), CC recommendations need to be crop-specific because of high inherent variability at the field-, farm-, or regional scales. Different crops can respond differently to the same CCs, even within the same farm. The development of a wider scientific knowledge base for the identification of CC niches in Canada warrants greater attention than has been accorded to date. To provide holistic recommendations, CC research needs to be tailored for specific regions, adaptive, and cost-conscious (Macrae et al., 2021). The costs of CC inclusion should be included in CC evaluations, as farmers will tend to gravitate towards cost-effective practices, and not necessarily towards the most environmentally beneficial (Bergtold et al., 2017). I assert that to effectively contribute to the widespread adoption of CCs, research objectives should be clearly defined in the context of the management system and should include the determination of the most cost-effective CC species or mixtures. It is critical that optimal management strategies in the context of specific cropping systems e.g., seeding and termination methods be added to basic scientific research. The identification of direct potential economic benefits of CCs could also go a long way in promoting adoption.

Research is essential for guiding policy, designing farm management strategies, promoting adoption of sustainable practices, and modifying future research protocols and priorities. In Canada, CC research is still in its infancy, and researchers often rely on studies from other regions in the US to guide their protocols. With increased local research on CCs, important progress is being made as some research findings are exploring ways to improve sampling protocols within the Canadian context. Future research can build on the findings of this study, either in the planning of experiments or formulation of research questions for similar or other cropping systems. Specifically, more CC species and mixtures need to be assessed for their potential to establish and contribute to soil organic C (and soil health) in corn systems. As more research is conducted, data will become available that will enhance our understanding of the most efficient strategies for CC inclusion in different regions and cropping systems.

Some important lessons that were highlighted in this research include:

- Conducting longer-term studies in which CC are an established part of the cropping system
- Assessment of more diverse groups of CCs and CC mixtures
- Implementation of sampling protocols that offer variable degrees of precision to account for variable target response variables
- Evaluation of parameters with a broader range of sensitivity, e.g., active C, PLFAs, or microbial DNA sequencing
- Assessment of both aboveground and belowground biomass interactions with soil processes
- Application of more robust statistical procedures e.g., multi-compartment models or mechanistic models involving soil biological processes. The development and application of models as research tools to support empirical research and to provide additional insights (Archontoulis and Miguez, 2015; de Neergaard et al., 2002; de Sa Pereira et al., 2017). Some models that have been successfully applied include the DeNitrification-DeComposition (Jarecki et al., 2018); HYDRUS (Andrews et al., 2020); Root Zone Water Quality Model (Malone et al., 2020); DayCent (McClelland et al., 2021), or AquaCrop model (Tenreiro et al., 2021); as well as remote sensing techniques such as LANDSAT or C-band Radarsat-2 polarimetric SAR imagery (Liao et al., 2018).

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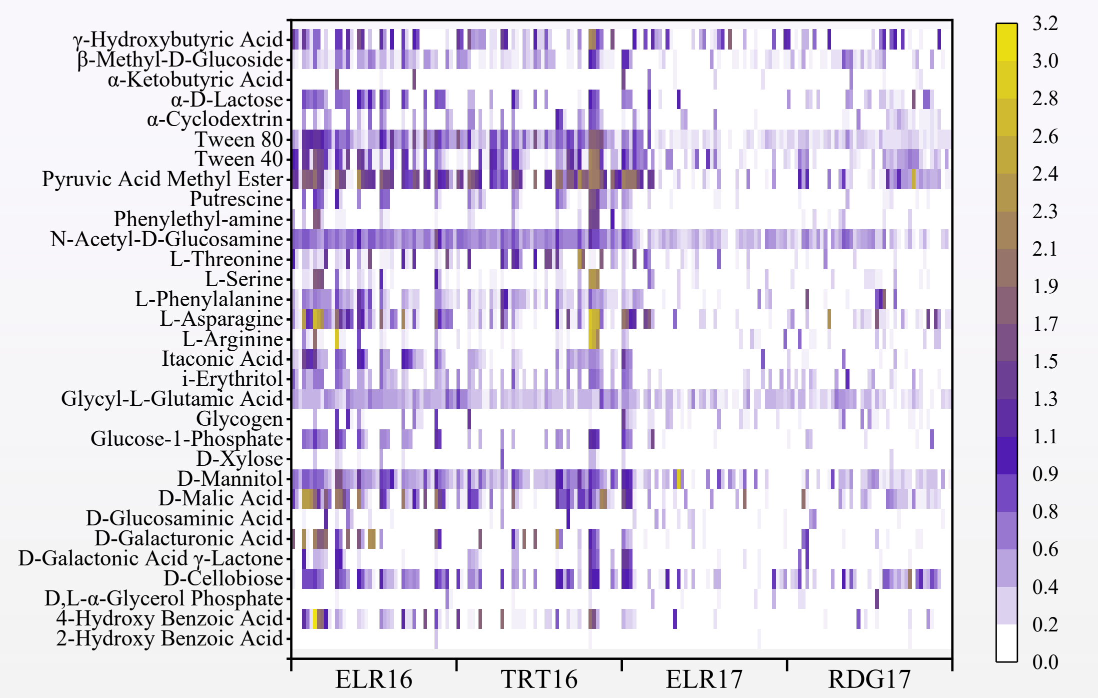
Appendix

Supplementary Table S0.1. Multilinear regression analyses for soil, corn, and cover crop attributes assessed in field study at three sites in Elora, Ridgetown, and Peterborough, ON

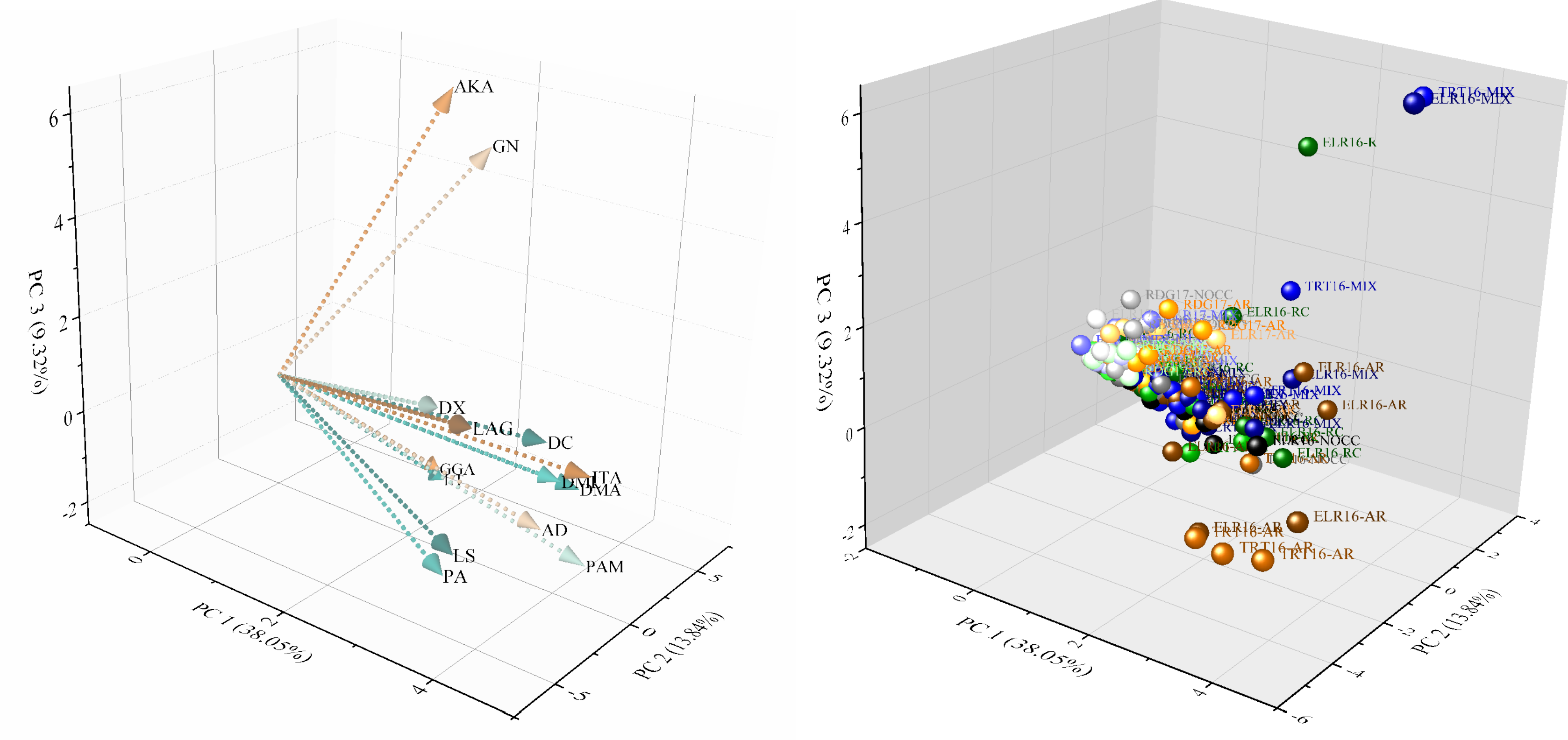
	Multilinear/Linear Regression†	**Error df**	**RMSE**	**R^2**
(i)	Grain Yield = 12.1 – 0.02 Fall Weeds‡	43	1.58	0.56
(ii)	Residual Soil N = -23.9 + 6.1 Stover N	43	7.47	0.52
(iii)	Spring SMN = -101 + 2.03 Grain N + 0.21 Grain C *Partial-R^2: Grain N = 0.45; Grain C = 0.14*	42	4.66	0.59
(iv)	Fall Cover Crop Biomass = 7.69 – 6.3 Grain C – 11.1 Stover C *Partial-R^2: Grain C = 0.22; Stover C = 0.30*	42	0.13	0.53
(v)	Spring Cover Crop Biomass = 0.77 + 1.69 Fall CC + 1.81 Fall Weeds *Partial-R^2: Fall CC = 0.61; Fall Weeds = 0.13*	42	0.26	0.74
(vi)	Total Cover Crop Biomass = 0.45 + 3.69 Fall Weeds	43	0.50	0.42
(vii)	Total Cover Crop C = 159 + 1.31 Fall Weeds	43	181	0.41
(viii)	Total Cover Crop N = 9.4 + 0.11 Fall Weeds	43	15.8	0.40

† All regression equations are significant at $P < 0.0001$; stepwise selection method used

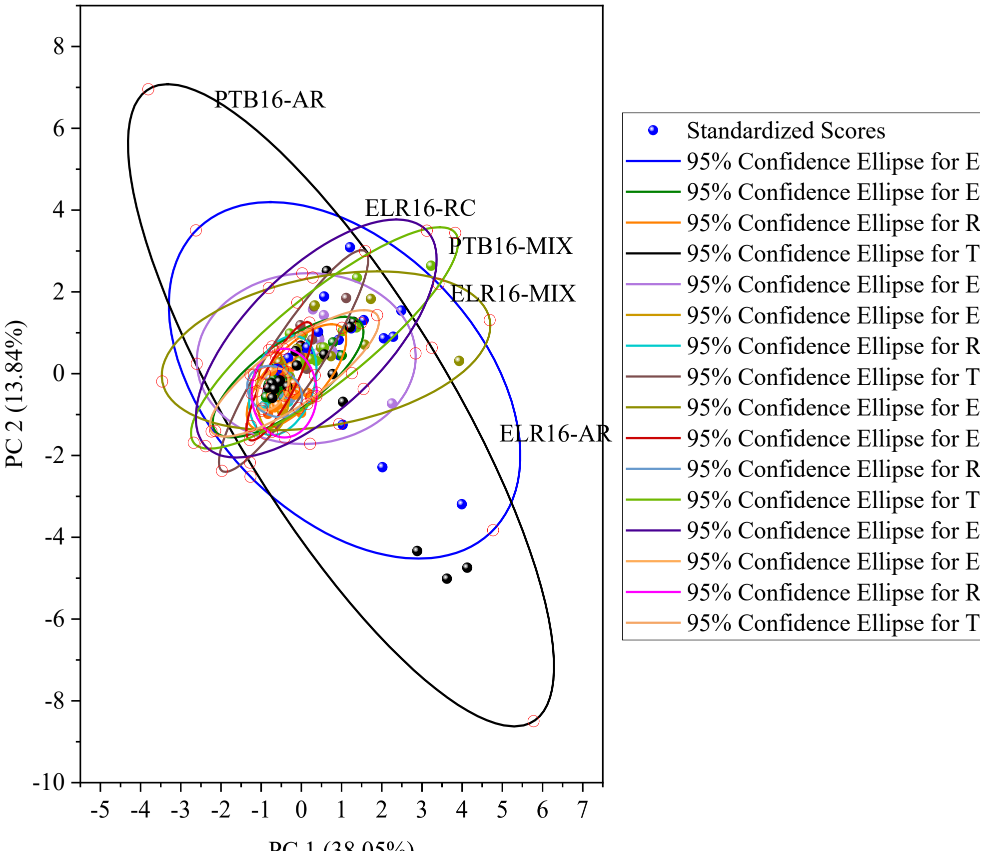
‡ All regression models were plotted, except those involving weeds as the as the only significant regressor



Supplementary Figure S1. Heatmap for standardized OD values for the 31 C sources on the BIOLOG EcoPlates™ used to assess effects of cover crops on microbial community level physiological profiles.



Supplementary Figure S1. Loading plot (a) and scores plot (b) of BIOLOG data showing interactive effects of site-year and cover crops. ELR = Elora, PTB = Peterborough, RDG = Ridgetown, AR = annual ryegrass, RC = red clover, MIX = AR + RC, and NOCC = no cover crop/control.



Supplementary Figure S2. Biplot (a) of BIOLOG Ecoplate data for annual ryegrass, red clover, CC mixture, and NOCC treatments applied to soil at Elora (ELR), Peterborough (PTB), and Ridgetown (RDG) in the 2015-16 and 2016-17 corn-growing seasons.