1. **ADF project details**

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4. Non-confidential summary

The overall study consisted of a series of bench- and greenhouse-scale studies undertaken to test the factors thought to contribute to N_2O emissions from canola, flax, pea, and wheat residues. Plant materials (residues) dually labeled with ^{15}N and ^{13}C were produced during Phase I of the study so that we could track residue-derived N and C during subsequent experiments. In the first experiment, soil was amended with crop residues that had been finely ground to eliminate particle size effects, and then incubated at room temperature and a soil water content that equally promoted nitrification and denitrification (i.e., 60% WFPS). Whereas residue-derived CO_2 emissions were directly related to the amount of residue-C added to the microcosms, there was no significant correlation between the amount of residue-N added and residue-derived N_2O. Molecular techniques showed that residue addition had no significant effect on the abundance of nitrification genes (A- and B-*amoA*), but had a significant influence on denitrification genes (primarily *nirS* and *nosZ*). Positive correlations between *nirS* gene abundance and N_2O emissions suggests that soil incorporation of crop residues promotes N_2O production. However, a negative correlation between N_2O emissions and *nosZ* gene abundance—the gene that codes for the enzyme that reduces N_2O to N_2—was also observed in the canola-amended microcosms. Thus, while all residues promoted N_2O production, canola residue also inhibited N_2O consumption thereby increasing the 'yield' of N_2O emitted (i.e., N_2O produced per unit of N turned over).

The second experiment involved spiking fertilized soil with freshly ground seed meal that either did (rapeseed) or did not (pea) contain glucosinolates. Results demonstrated that one or more glucosinolate-derivatives strongly impacted microbial N processing dynamics in such a way as to produce a dramatic increase in N_2O emissions. Moreover, there was a strong interaction between fertilizer-N addition and the incorporation of glucosinolate-containing seed meal. This finding, together with those of the first experiment, strongly suggest that one or more of the derivatives of glucosinolate decomposition "turns off" *nosZ* gene expression, resulting in a concomitant increase in N_2O yield.

The third experiment involved a greenhouse study in which wheat was grown in soils amended with canola, flax or wheat residues and fertilizer-N. Total N_2O emissions in the planted mesocosms exhibited no significant treatment (residue) effect, while residue-derived emissions from the mesocosms amended with canola residue were lower than those amended with wheat residue. The presence of the growing plants would have competed for N and kept the soils drier (more aerated)– conditions that would disfavor denitrification (the process most influenced by glucosinolates). As a result, residue-induced N_2O emissions were more influenced by the rate of N release from residues (wheat turning over more quickly than canola).

5. Introduction

Retailers and consumers of agricultural products are increasingly concerned about the environmental impact of these products, and there is a trend among retailers to require detailed accounting of "carbon footprinting" along a supply chain. For example, Walmart, the world's largest grocery retailer, launched *Project Gigaton* in 2016 with the intent of reducing GHG emissions along its supply chain by 1 Gt by 2030. This would include a reduction of 300 Mt of GHG emissions from the agriculture sector through the adoption of "best-in-class" management practices. In order to meet these goals, all components of the agri-food supply chain (including producers) must be able to (i) provide reliable estimates of their emissions, (ii) demonstrate measurable improvements, and (iii) improve soil health. For field crops, nitrous oxide (N_2O) emissions are an important component of their overall carbon footprint and, therefore a key sustainability indicator.

Agricultural soils are a major source of nitrous oxide (N_2O), a potent greenhouse gas with a global warming capacity about 300-times greater than that of carbon dioxide (CO_2). Based on current IPCC (International Panel on Climate Change) methodologies, estimates of N_2O emissions in Canada indicate that 24% of all agriculture-based emissions are associated with the use of N fertilizers and that another 17% are associated with the decomposition of crop residues. On the one hand, crop residues are important to the overall sustainability of cropping systems. They provide carbon to maintain or improve soil organic carbon balance, and as residues decompose they release nutrients to soil, thereby supporting soil fertility. However, these nutrients also provide substrate for microbial activity. Nitrogen, in particular, serves as a substrate for nitrification and denitrification, which are the primary mechanisms for the production of soil-derived N_2O. Residues produced by different plant species decompose at different rates, and hence affect plant uptake and yield, and GHG emissions differently. Furthermore, little information is available regarding the influence of residue type and their potential interactions with fertilizer N applications. However, research examining N_2O emissions associated with decomposing crop residues is extremely limited. Several studies have demonstrated a link between the C:N ratio of a crop residue and the magnitude of residue-induced N_2O emissions (Begum et al. 2013; Chen & Li, 2013; Shan & Yan, 2013), with high C:N ratio (>20:1) residues generally yielding lower emissions due to microbial immobilization of N in the soil. However, residue decomposition, N mineralization and N_2O production are also affected by factors such as chemical composition of the residue, soil type, and climate.

A recently completed bench-scale incubation study funded by ADF and the Saskatchewan Pulse Growers (20100190) examined the effects of soil type and water content on N_2O emissions associated with the decomposition of different crop residues (Farrell et al. 2014). Nitrification and denitrification both produce N_2O as one of the end products, but the processes are influenced by different climate conditions. For example, nitrification is an aerobic process, requiring oxygen and hence occurs in drier soils, whereas denitrification is an anaerobic process, occurring in oxygen restricted conditions, and hence occurs in wetter soils. Thus the study was carried out using soils from the Black, Dark Brown, Brown, and Gray soil zones and was conducted under conditions favoring nitrification [i.e., at 50% water-filled pore space (WFPS)] or denitrification (i.e., at 70% WFPS). The soils were amended with ^{15}N-labeled residues of wheat, pea, canola, and flax; and $^{15}N_2O$ production was monitored during a 5-wk incubation. Whereas both water content and soil type had an impact on N_2O production, there was a clear and consistent trend in the emission factors for the residues; i.e., emission factors (N_2O-N emitted expressed as a percentage of total residue-N) increased in the order: wheat (1.13) ≤ urea (1.33) < pea (2.83) ≤ flax (3.43) << canola (6.49). The same pattern of higher emissions from canola and flax compared to the other treatments was observed for both N_2O from nitrification and denitrification, but the amounts were much higher (*ca*. 15×) from denitrification. NOTE: these are not IPCC emission factors, but

rather percentage losses of N_2O relative to the total amount of N in the residue. These amounts are significant not only because of the global warming potential of the N_2O, but also because they represent a loss of N from the system; N that is not available for crop growth and yield. Results of this research demonstrated that—under the right environmental conditions—there is considerable potential for N_2O emissions from decomposing crop residues, and that this appears to be especially true for oilseed (canola and flax) residues. Results from these experiments are supported by results from a recent field study reported by Lemke *et al.* (2018), who reported higher N_2O emissions from wheat grown on canola residue than wheat grown on pea or wheat residues. However, before mitigation options can be explored it is first necessary to determine the factors contributing to the high emissions associated with the oilseed residues.

The overall goal of the research reported here, was to identify the factors contributing to previous observations that nitrous oxide (N_2O) emissions from canola and flax residues were greater than those from wheat residues. Information from the project will identify specific factors that influence nitrous oxide emissions from residue decomposition, and help point the way towards potential mitigation strategies that can be incorporated into Best Management Practices.

6. Methodology

6.1 Overview of Study

The overall study consisted of a series of bench- or greenhouse-scale studies undertaken to test the factors thought to contribute to N_2O emissions from flax and canola residues.

Potential reasons that N_2O emissions from canola and flax residues can be higher than those from wheat or pea residue include: (i) increased N availability due to higher mineralization or lower immobilization rates in oilseed compared to cereal or pulse residues; (ii) increased dissolved organic carbon (DOC) status resulting from higher decomposition rates due to compositional (proportions of lignin, cellulose, hemicellulose, sugars, starches etc.) differences in the residues of oilseed compared to cereal or pulse residues; (iii) differences in residue decomposition rates resulting in increased oxygen consumption causing greater anaerobic conditions and higher denitrification activity; (iv) differences in residue decomposition resulting in differing microbial substrate ratios (i.e., available N:available C) which, in turn, modify the end product ratios (N_2O:N) of denitrification; and (v) the presence of residue-specific compounds (allelopathic chemicals) that stimulate or inhibit components of the microbial population. For example, heterotrophic activity may be inhibited allowing nitrifiers to more effectively compete for available NH_4 which, in turn, can be converted into N_2O.

Phase I (preparation of ^{15}N and ^{13}C labeled plant residues). Canola, flax and wheat plants were grown in the greenhouse and dually labeled with ^{15}N and ^{13}C. A complete characterization of the residues was conducted—including fiber content and quality [i.e., dry matter, crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent insoluble crude protein (ADICP)], as well as the proportions of lignin, cellulose, and hemicellulose and their respective profiles. In addition, the residues were analyzed for compounds thought to be allelopathic and that may affect microbial populations—e.g., glucosinolates, lipids (linoleic & alpha-linoleic acids) and cyanogenic glycosides.

Soil was collected from unfertilized research plots at the University of Saskatchewan Goodale Research Farm. The soil had a loam texture, a pH of 7.6, and an organic matter content of 1.7%; soil test results (*ALS Laboratory Services*, Saskatoon) indicated that the soil was low in N (12 kg NO_3-N ha^{-1}), P (16 kg P ha^{-1}) and S (11 kg SO_4-S ha^{-1}), but had adequate K (436 kg K ha^{-1}). To ensure that the added ^{15}N-labeled fertilizer was used efficiently,

and to facilitate recovery of the plant roots at harvest, the soil was mixed with sand (60:40 soil:sand, w/w) and approximately 1.6 kg of the soil-sand mixture placed in each of 16 plastic containers (60-cm × 40-cm × 30-cm; l×w×d). Each of the four crops [wheat (AC Barrie), canola (Dekalb 72-65 RR), flax (cv. CDC Bethune), and pea (cv. CDC Treasure)] were then seeded into replicate (n = 4) containers and placed on a tented bench in the greenhouse.

One week after seedling emergence, the plants received 250-mL of a fertilizer solution prepared by dissolving 127.5-g Miracle Grow® All Purpose (24% N) plant food, 68-g K_2SO_4 and 0.69 g ^{15}N-urea (98 atom% ^{15}N) in 8.5 L of distilled water. The fertilizer solution contained an excess (1 atom%) of ^{15}N, and each container received the equivalent of 75 kg N ha^{-1}, 25 kg P_2O_5 ha^{-1}, 115 kg K_2O ha^{-1}, and 30 kg S ha^{-1}. The fertilizer solution was added to the soil between the plant rows (making sure not to get fertilizer solution on the plant leaves) and washed into the soil using additional increments (2 L) of water. Soil moisture was monitored using a Rapitest-mini Soil Moisture Meter (Luster Leaf® Products, Inc.; Woodstock, IL) and was maintained at 75 ± 5% of field capacity throughout the growing season.

The plants were labelled with ^{13}C by introducing an excess of ^{13}C-labeled CO_2 (*Cambridge Isotope Laboratories, Inc.*; Tewksbury, MA) into the atmosphere inside the tent and allowing the plants to take up the $^{13}CO_2$ during photosynthesis. To accomplish this, the tent (1.5-m × 4.3-m × 1.4-m; h×l×w; V = 9.03 m^3) was closed and a known volume (0.2 L to 0.6 L) of $^{13}CO_2$ (99 atom%) injected into the enclosed atmosphere[1]. The CO_2 concentration in the enclosed atmosphere was monitored using a CAP PPM-3 CO_2 Monitor/Controller and when the CO_2 concentration dropped below the lower set-point (200 ppmV), the tent was opened and remained open until the CO_2 concentration reached ambient levels in the greenhouse (*ca*. 502 ± 70 ppmv); the tent was then closed and the labeling procedure repeated. The labeling procedure was carried out twice per week—beginning two weeks after plant emergence and ending approximately two weeks prior to harvest.

Plants were harvested when they reached maturity (11–15 weeks after emergence depending on the crop) and, at harvest, the seed was separated from the above-ground biomass and both the seed and remaining straw residue (i.e., leaves + stems + pods) were dried at 60°C to a constant weight. Roots were carefully removed from the soil-sand mixture using a 2-mm sieve and tweezers; washed on a 0.5-mm sieve with deionized water; and dried at 60°C to a constant weight. The straw and roots were then coarsely ground using a coffee grinder, and sub-sampled—with the subsamples being finely ground using a ball mill. The finely ground plant samples were then analyzed using isotope ratio mass spectrometry (IRMS; Delta V Advantage, Isomass Scientific Inc.; Calgary, AB) to determine total N, total C, ^{15}N, and ^{13}C. In addition, sub-samples of the coarsely-ground plant materials were submitted to (i) the AAFC Saskatoon (Dr. Tim Dumonceaux) and Swift Current (Dr. Allan Iwassa) Research & Development Centers for fiber content and quality assessment (including lignin, cellulose and hemicellulose content); and (ii) the *Lipid Quality & Utilization Laboratory* (Dr. Martin Reaney) in the Department of Plant Sciences for more detailed biochemical profiling (including glucosinolates, lipids, and cyanogenic glycosides).

Phase II: consisted of short-term microcosm incubations designed to answer the following questions:

1) *Do the physical characteristics of the residue influence nitrous oxide emissions?*

[1] **Note:** the amount of CO_2 added was increased as the rate of photosynthesis increased (i.e., as the plants became larger). Fans placed inside the tent were used to ensure that the $^{13}CO_2$ was thoroughly mixed and homogenously distributed throughout the tent.

2) *Does the biochemical composition of the residue influence N_2O emissions?*

3) *Are there elements within the residues that influence N_2O emissions?*

To answer these questions, one set of soil microcosms was amended with residues of flax, canola, and wheat that were finely ground to provide homogenously sized crop materials (i.e., provide residues with a more uniform surface area distribution), thereby eliminating the influence of differences due to physical breakdown of the materials. A second set of soil microcosms was amended with glucosinolates[2] (GSLs) or phenylethyl-isothiocyanate (*p-ITC*), which are more abundant in oilseed crops than cereal crops, and are known to have both stimulatory and inhibitory effects on soil microbial communities (Snyder et al., 2010), N mineralization (Bending & Lincoln, 2000), and nitrification (Brown & Morra, 2009).

Residue-amended soil incubation. Soil for the microcosm studies was collected from the research plots used in the Canola-N study (*ADF Project No. 20130130*) at the Canada-Saskatchewan Irrigation Diversification Centre (CSIDC) in Outlook, SK. The soil was transported to the *Prairie Environmental Agronomy Research Laboratory (PEARL)* in the Department of Soil Science where it was air dried and screened to pass a 2-mm sieve and remove any visible plant residue, and sent to *Farmers Edge Laboratories* (Winnipeg, MB) for analysis. The soil had a sandy loam texture, a pH of 7.5, EC of 0.86 dS cm^{-1}, and an organic matter content of 2.8%; soil test results indicated that the soil was high in available N (119 kg NO_3-N ha^{-1}), P (78 kg P ha^{-1}), K (516 kg K ha^{-1}), and S (36 kg SO_4-S ha^{-1}). Total organic C and total N content of the soil (and residues) were determined in the Department of Soil Science, using a standard dry-combustion technique (Skjemstad & Baldock, 2008; Rutherford et al., 2008).

The treatments included a blank (no soil and no residue; used to obtain $\delta^{15}N$ values for the laboratory air); a control (soil with no residue; used to determine background emissions); and soil amended with one of four crop residues (canola, flax, pea, or wheat). Each treatment combination was replicated four times[3], with the microcosms arranged using a completely randomized design. Prior to the start of the microcosm study, the soil was brought to (and maintained at) a gravimetric soil water content (GSWC) of 17% (approximately 55% water-holding capacity) to allow the soil microbial community to stabilize—during a three week pre-incubation period at room temperature—and eliminate the CO_2-flush that occurs on rewetting of a dried soil (Lee et al., 2000). At the end of the pre-incubation period, the $^{15}N/^{13}C$-labelled crop residues were incorporated into the soil at rates based on previous studies (Farrell et al., 2014, 2017) (see Table 1). All treatment replicates were prepared individually by adding the residue (above- and below-ground residue) to an appropriate amount of moist soil (i.e., equivalent to 135 ± 0.5 g oven-dry weight) in the stainless steel bowl of a Cuisinart® 5.5 Qt. Stand Mixer; the sample was then homogenized by mixing (speed setting = 6) for two minutes. The residue-amended soil was then transferred into a 40-dram plastic vial, packed to a bulk density of 1.2 g cm^{-3}, wetted with enough water to bring the final water content to 60% water-filled pore space (WFPS), and placed in a 1-L Kilner jar that was sealed with a polypropylene lid fitted with a self-sealing septum to allow for gas sampling. The jars were then incubated in the dark at room temperature (24 ± 2°C) for *ca*. six weeks.

Gas samples were collected throughout the 49-d incubation period, but were sampled with greater frequency at

[2] Note: the proposal indicated that the microcosms would be amended with "specific glucosinolates, cyanogenic glycosides, or linoleic and alpha-linoleic acids"; however, these compounds were not detected in the crop residues during the biochemical analyses performed by Dr. Reaney's lab (likely because they were present at concentrations below the analytical detection limits of the equipment) and the cost of further, more sensitive analyses was prohibitive.

[3] An additional set of "destructively sampled" microcosms (n = 48: 6 trt × 4 sampling times × 4 reps) was set up for microbial analyses.

the start of the experiment; i.e., at 4, 18, 24, 36, 48, 96, 145, 193, 242, 290, 362, 283, 604, 727, and 971 h after incorporation of the residues. Headspace samples were collected from the microcosms through a sampling port consisting of a gray butyl rubber septum sealed into the lid, and were collected using a 30-cc gas tight syringe[4,5]. The gas samples were immediately injected into pre-evacuated 22-mL Kimax™ glass vials (Fisher Scientific, Ottawa, ON) for analysis. All samples were analyzed for total GHG concentrations using gas chromatography (SCION 456 GC equipped with a ^{63}Ni electron capture detector [for N_2O] and thermal conductivity detector [for CO_2]; SCION Instruments, Edmonton, AB) (Farrell & Elliott, 2007; David et al., 2018); isotopic gas analyses were conducted using Picarro isotopic $^{15}N_2O$ and $^{13}CO_2/^{13}CH_4$ analyzers (G-5131-*i* and G-2201-*i*, respectively; Picarro Inc., Santa Clara, CA) (Congreves et al., 2019; Farrell et al., 2019). Nitrous oxide concentrations in the headspace often exceeded the upper limit of the Picarro G5131-*i* analyzer (i.e., 2 ppmv); consequently, the total N_2O concentration in the headspace gas was first determined using gas chromatography. Samples containing >2 ppmv N_2O were then diluted with *zero-air* and run on the Picarro G5131-*i* analyzer to obtain the isotopic signature ($\delta^{15}N$) of the N_2O.

Table 1. Residue additions to the soil microcosms.[a]

Residue		Residue-N		Total N addition		Residue-C		Total C addition	
(crop)	**(mg)**	**(%)**	**(atom% ^{15}N)**	**(mg N)**	**(μg ^{15}N)**	**(%)**	**(atom% ^{13}C)**	**(mg C)**	**(mg ^{13}C)**
					Above-ground residue (AGR)				
Canola	866.6	1.09	0.6283	9.40	59.06	43.06	1.2729	373.15	4.75
Flax	541.3	1.74	0.5640	9.44	53.24	46.23	1.2577	250.25	3.15
Pea	675.1	1.10	0.5718	7.45	42.60	42.90	1.2585	289.64	3.65
Wheat	674.9	0.87	0.5885	5.89	34.66	42.06	1.2333	283.86	3.50
					Below-ground residue (BGR)				
Canola	219.8	0.86	0.5515	1.90	10.48	42.66	1.1928	93.77	1.12
Flax	41.2	0.91	0.5147	0.37	1.90	43.05	1.1972	17.74	0.21
Pea	130.8	1.91	0.5161	2.49	12.85	41.65	1.1681	54.48	0.64
Wheat	78.8	0.81	0.4993	0.64	3.20	43.91	1.1780	34.60	0.41

[a] Oven-dry weight of soil added to microcosms = 135 g.

Daily N_2O (total and $^{15}N_2O$) and CO_2 (total and $^{13}CO_2$) fluxes were plotted versus elapsed time and cumulative emissions were calculated using an area-under-the-curve (AUC) analysis. Concentrations of residue-derived ^{15}N and ^{13}C were calculated from the isotopic data, which together with the GC data were used to calculate the total amounts of residue-derived N_2O and CO_2 for each treatment combination; residue-induced emissions were calculated by correcting for background emissions.

[4] Syringes used to sample the microcosms containing $^{15}N/^{13}C$-labeled residues were not used to sample the blank or control [non-labeled residue] microcosms.

[5] To eliminate pressure deficit effects, an equivalent amount of certified *zero air* was injected into the headspace of the microcosm after removal of each gas sample; dilution effects were accounted for when calculating the total N_2O concentrations.

Soil microbial abundance and functional group analysis. Once gas sampling was completed, microbial community analyses were conducted using randomly selected microcosms that were destructively sampled after the 0, 48, 97 and 362 h gas samples were collected. Abundance and diversity of ammonia oxidizers and denitrifiers were measured by qPCR.

Bacterial and archaeal ammonia oxidizers and denitrifier community composition of fresh soil were analyzed with qPCR. Soil DNA was extracted from soil sampled by weighing 0.25 g of processed stored soil using PowerSoil DNA isolation kit and following the manufacturer's instructions (MO BIO Laboratories, Inc., CA). DNA extracts was stored at -80°C (Smith et al., 2010), and quantified using qPCR, with appropriate primers to target key genes in the nitrification and denitrification pathways (archaeal *amoA*, bacterial *amoA*, *nirS*, *nirK* and *nosZ*) (Helgason et al., 2018).

Data analyses were performed using IBM SPSS ver. 25. A linear mixed model for repeated measurement was used to analyze the significance of differences between labeled residue applications on: N_2O emissions (total and $^{15}N_2O$), CO_2 emissions (total and $^{13}CO_2$), bacterial and archaeal ammonia oxidizers and denitrifier community composition at the $P \leq 0.05$ level of probability. Non-metric multidimensional scaling (NMS) using PCOrd v.5.10 (MjM Software Gleneden Beach, OR) was used to analyze the community composition of the PLFA data.

Glucosinolate-amended soil incubation. Soil (0–10 cm) for the incubation study was collected from the organic plot at the AAFC Scott research station. Prior to the start of the incubation study, the soil was air dried and sieved to pass a 4-mm screen to remove stones and any visible pieces of plant debris. Gravimetric water content was determined after oven drying at 105°C for 24 hours; soil moisture was then adjusted to (and maintained at) 25% GSWC (equivalent to 60% WFPS), and stored at 23°C for 14 days to rejuvenate and stabilize the soil microbial community (Lee et al., 2000). After the pre-incubation period, moist soil (25.89 g oven dry equivalent) was weighed and packed into small plastic Petri dishes (5.8-cm i.d. × 0.8 cm tall) to a bulk density of 1.2 g cm^{-3}, and the gravimetric soil water content adjusted to 0.27 g H_2O g^{-1} soil (i.e., equivalent to 65% WFPS). The soils were then placed in 1-L Kilner jars fitted with gas tight lids, placed in an incubator using a completely randomized design, and maintained in the dark at 23°C for 23 days. The individual microcosms were maintained at 65% WFPS by adjusting the gravimetric soil water content as needed. A total of 45 microcosms were prepared: (9 treatments × 5 replicates).

The treatments included a blank (i.e., no soil and no residue) used to obtain background gas concentrations; non-fertilized and fertilized controls[6] used to establish baseline (i.e., soil-derived) N_2O and CO_2 emissions; and seven amendment combinations: (i) soil + N; (ii) soil + N + high concentration GSL[7] (H-GSL); (iii) soil + N + intermediate concentration GSL (I-GSL); (iv) soil + N + low concentration GSL (L-GSL); (v) soil + N + *p-ITC*; (vi) soil + N + pea meal; and (vii) soil + N + pea meal + *p-ITC*. The treatments were applied to supply C and N as shown in (Table 2).

Gas samples were collected throughout the 23-d incubation period, but were sampled with greater frequency at the start of the experiment. Headspace samples were collected from the microcosms through a sampling port consisting of a gray butyl rubber septum sealed into the lid. Headspace samples were collected using a 35 mL

[6] Nitrogen was added as 1.0 atom% ^{15}N-labeled urea (0.71 mg N per microcosm).

[7] **Note:** the GSLs were added as freshly ground rapeseed meal containing known amounts of total glucosinolates (Sigma-Aldridge, Oakville, ON). H-GSL = 99 μmol g^{-1} seed; L-GSL = 11.9 μmol g^{-1} seed; I-GSL = 1:1 (w/w) blend of H-GSL and L-GSL.

gas-tight syringe. (Note: syringes used to sample the microcosms containing ^{15}N urea were not used to sample the blank or control [non-labelled] microcosms, and the syringes were flushed with ultra-zero air between treatments). On each sampling date, gas samples were collected from each microcosm and transferred to a pre-evacuated 12-mL Exetainer vial (Labco Inc.; Ceredigion, UK). Then used to determine the total concentrations of N_2O and CO_2 in the headspace samples using gas chromatography (David et al., 2018), and were then used for isotopic analysis of the headspace gas using cavity ringdown spectroscopy (CRDS; Picarro G2201-*i* isotopic CO_2 / CH_4 analyzer and G5131-*i* isotopic N_2O analyzer; Picarro Inc., Santa Clara, CA). Total concentrations of N_2O and CO_2 in the headspace gas were determined using a SCION 456GC gas chromatograph equipped with an electron capture detector (ECD) for the determination of N_2O and a thermal conductivity detector (TCD) for the determination of CO_2. If necessary (i.e., depending on the total gas concentration), samples collected for isotopic analysis were diluted with ultra-zero-air before analysis for $^{15}N_2O$. After the last sample was collected, each microcosm was placed in a fume hood and left open for 20 min to replenish the atmosphere with ambient air.

Table 2. Soil amendment combinations used to investigate the effect of glucosinolate and its derivatives on soil N_2O and CO_2 emissions.

Treatment ID[a]	C source (mg C microcosm^{-1})				N source (mg N microcosm^{-1})			
	Soil	Seed meal	Fertilizer	Total	Soil	Seed meal	Fertilizer	Total
Control (soil)	699.03	- - -	- - -	699.03	64.73	- - -	- - -	64.73
N	699.03	- - -	0.31	699.34	64.73	- - -	0.71	65.44
N + *p-ITC*	699.03	0.34	0.31	699.68	64.73	- - -	0.71	65.44
N + H-GSL	699.03	18.77	0.31	718.11	64.73	1.18	0.71	66.62
N + I-GSL	699.03	18.77	0.31	718.11	64.73	1.08	0.71	66.52
N + L-GSL	699.03	18.77	0.31	718.11	64.73	0.97	0.71	66.41
N + Pea Meal	699.03	18.77	0.31	718.11	64.73	1.52	0.71	66.96
N + Pea Meal + *p-ITC*	699.03	19.11	0.31	718.11	64.73	1.52	0.71	66.96

[a] C_{AI} = concentration of the active ingredient: *p-ITC* = phenyl-isothiocyanate (0.12 μmol g^{-1} soil); GSL = glucosinolate; H = high concentration (124 nmol GSL g^{-1} soil), I = intermediate concentration (69.31 nmol GSL g^{-1} soil), and L = low concentration (14.88 nmol GSL g^{-1} soil).

Phase III: consisted of a greenhouse study in which ^{13}C- and ^{15}N-labeled residues (prepared in Phase I) were applied to soil mesocosms seeded with wheat (AC Carberry). Residues labeled with ^{13}C and ^{15}N were used to differentiate between N_2O and CO_2 generated from the residues (with the label) and those generated from the soil or fertilizer (without the label). The amounts of residue (canola, flax, and wheat) applied were selected to provide equivalent rates of N (i.e., 445 mg residue-N pot^{-1}). Spring wheat was planted in each of the soil mesocosms, which were fertilized with urea (915 mg urea-N pot^{-1}; equivalent to 50 kg N ha^{-1}). The experiment was conducted in a greenhouse under conditions approximating field conditions.

Soil for the mesocosm study was collected from a low-N field site at the U of S Goodale Research Farm (Saskatoon, SK) and returned to the *PEARL* where it was air dried, sieved (<2 mm) to remove any rocks, and

mixed (60:40 w/w) with coarse sand to ensure that the added ^{15}N-enriched residue was used efficiently and facilitate recovery of the plant roots at harvest. Sub-samples of the soil:sand mix were sent to *Farmers Edge Laboratories (Winnipeg, MB)* for analysis. The soil had a loamy sand texture, a pH of 7.8, EC of 0.43 dS cm^{-1}, and an organic matter content of 2.5%; soil test results indicated that the soil was high in available N (47 kg NO_3-N ha^{-1}), P (42 kg P ha^{-1}), K (695 kg K ha^{-1}), and S (40 kg SO_4-S ha^{-1}). Approximately 5-kg of the soil-sand mixture was placed into specially designed pots that allowed the gas samples to be collected without disturbing the plants (Fig. 1). Initially, the soil water content was adjusted to 50% WHC and the pots equilibrated in the greenhouse for one week to allow the soil to settle. The crop residues were then incorporated into the surface 5-cm of the soil in replicate (n = 6) containers; wheat (AC Carberry) was seeded into the containers as shown in Fig. 1, and the soil water content adjusted to 55% WHC.

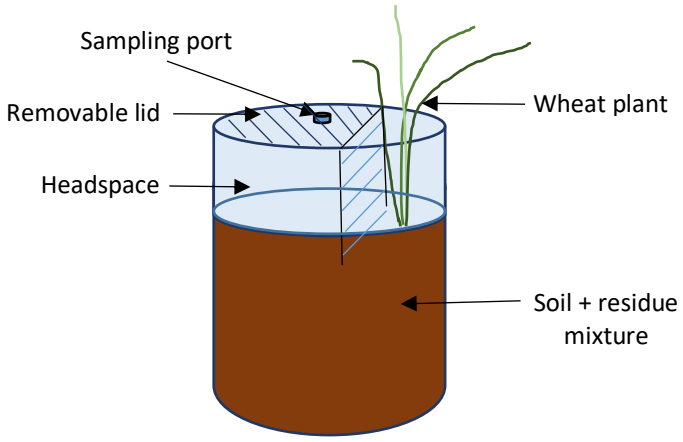

Figure 1. Schematic of the pots (60-cm × 40-cm × 30-cm; h×w×d) used for greenhouse gas (N_2O) sampling during the wheat phase of the experiment.

Greenhouse gas (N_2O) sampling was initiated 24-h after seeding and continued throughout the growth period, terminating at harvest. On each sampling date, a lid was attached to the pot and a single headspace sample was collected at t = 60 min. Two gas samples were collected using a 30-cc syringe equipped with a 22-gauge needle, and were withdrawn through a sampling port in the lid (see Fig. 1). Each gas sample was injected into a pre-evacuated 12-mL Exetainer vial; one sample was analyzed for total N_2O concentration using gas chromatography (SCION 456GC equipped with a ^{63}Ni electron capture detector) while the second gas sample was analyzed for $^{15}N_2O$ concentration using cavity ring-down spectroscopy (CRDS; Picarro G5301-*i* isotopic N_2O analyzer). Daily N_2O fluxes (mg N_2O-N m^{-2} d^{-1}) were plotted versus elapsed time (d) and cumulative emissions calculated using an area-under-the-curve (AUC) analysis. The total cumulative N_2O-N and cumulative $^{15}N_2O$-N emission data were used to calculate emission factors (EF) for the different crop residues.

Statistical Analyses

Greenhouse gas fluxes—especially N_2O fluxes—are known to be highly variable with non-normal distributions. Thus, a preliminary data analysis was conducted to determine if the data were normally distributed (using the Shapiro-Wilk test) and test for homogeneity of variance (using Bartlett's test). Data that was not normally distributed was transformed (using a Log_{10} transformation) prior to analysis of variance (ANOVA).

Transformed data was then back-transformed for presentation in the figures and tables. Treatment effects were determined using a one-way ANOVA with contrasts using CoStat (version 6.4, CoHort software, Monterey, CA). Tukey's HSD was used to determine treatment differences and, unless otherwise noted, all analyses were conducted using a probability level of $\alpha = 0.05$.

6. Research accomplishments (Results and Discussion)

Phase I (preparation and characterization of ^{15}N and ^{13}C labeled plant residues)

All crops were harvested when they reached maturity; i.e., at *ca*. 11 wk after emergence for the canola and pea, and at *ca*. 14 wk after emergence for the flax and wheat. Seed yield was greatest for the wheat and pea, and lowest for the canola and flax (Table 3). The total N content of the seeds was greatest for the flax (4.51%) and canola (4.41%), intermediate for the pea (3.73%) and lowest for the wheat (3.37%). Despite having the highest total N content, the flax exhibited the least amount of ^{15}N-enrichment in the seed, whereas the canola exhibited the greatest amount of ^{15}N-enrichment in the seed. Overall, ^{15}N-enrichment of the seeds ranged from 0.2005 to 0.2713 atom% excess and increased in the order: flax < pea < wheat < canola ($P < 0.001$). In addition, ^{13}C-enrichment of the seeds ranged from 0.1776 to 0.2117 atom% excess and increased in the order: flax < wheat < canola < pea ($P < 0.001$).

Table 3. Seed yield, total C and ^{13}C content, and total N and ^{15}N content of the harvested seed[a].

Crop	Yield (g m^{-2})	(%C)	($\delta^{13}C$)	(atom% ^{13}C)	(%N)	(atom% ^{15}N)[b]	C:N
Wheat	192 a	44.7 c	155 c	1.2749 c	3.37 c	0.6008 b	13.2 a
Canola	67.8 b	58.3 a	160 b	1.2800 b	4.40 a	0.6376 a	13.2 a
Flax	26.4 b	56.2 b	140 d	1.2585 d	4.51 a	0.5668 d	12.5 b
Pea	181 a	45.0 c	171 a	1.2926 a	3.73 b	0.5717 c	12.1 b

[a] Within columns, means (n = 4) followed by the same letter are not significantly different ($P \leq 0.05$).

[b] Natural abundance concentrations = 1.0809 atom% ^{13}C and 0.3663 atom% ^{15}N.

Above-ground (AG) residue production (stems + leaves + pods) was greatest for the canola and flax, and least for the wheat and pea (Table 4). Total N content of the AG residues ranged from 0.87% in the wheat residues to 1.74% in the flax residues, with the canola and pea residues being intermediate (see Table 3). Not surprisingly, therefore, the C:N ratio of the AG residues was greatest for wheat (48:1) and lowest for flax (27:1). The AG residues of all crops exhibited significant ^{15}N and ^{13}C enrichments—ranging from 0.1977 to 0.2620 atom% excess ^{15}N and from 0.1524 to 0.1920 atom% excess ^{13}C. In general, ^{15}N enrichment of the AG residues followed the same pattern observed for the seeds; i.e., it increased in the order: flax < pea < wheat < canola ($P < 0.001$). On the other hand, ^{13}C enrichment of the AG residues followed a different pattern—increasing in the order: wheat < flax = pea < canola ($P < 0.001$).

Below-ground (BG) residue (root) production was greatest for the flax, canola and wheat, and least for the pea (Table 5). The total N content of the BG residues was generally lower than that of the AG residues—the lone exception being the pea, which exhibited an N content (1.91%) that was significantly greater than that found in

the AG residues (i.e., 1.10%). Whereas there was significant isotopic (^{15}N & ^{13}C) enrichment of the BG residues (see Table 5), total enrichment was generally lower than that of the AG residues—ranging from 0.1330 to 0.1852 atom% excess ^{15}N and from 0.0872 to 0.1163 atom% excess ^{13}C. As was the case with the seed and AG residues, canola exhibited the greatest amount of ^{15}N enrichment. Nitrogen-15 enrichment of the other BG residues, however, did not follow the pattern observed for the seed and AG residues; i.e., ^{15}N enrichment of the BG residues increased in the order: wheat < flax = pea < canola. The high total N content, and relatively low ^{15}N enrichment, of the BG pea residues presumably reflects the fact that the pea residue included both the roots and the root nodules where biological nitrogen fixation occurred—and which typically have relatively high N contents (Arcand et al., 2013). Carbon-13 enrichment of the BG residues was greatest for the oilseed crops and least for the pea.

Table 4. Carbon and nitrogen content, and isotopic (^{15}N & ^{13}C) composition of the above-ground (stems + leaves + pods) biomass.

Crop	AG[a]						C:N
	Biomass (g m^{-2})	(%C)	($\delta^{13}C$)	(atom% ^{13}C)	(%N)	(atom% ^{15}N)	
Wheat	248 c	42.1 c	117 c	1.2333 c	0.87 c	0.5885 b	48.1 a
Canola	574 a	43.1 b	153 a	1.2729 a	1.09 b	0.6283 a	39.7 b
Flax	414 b	46.2 a	139 b	1.2585 b	1.74 a	0.5640 d	26.6 c
Pea	214 c	42.9 b	140 b	1.2585 b	1.10 b	0.5718 c	39.0 b

[a] Within columns, means (n = 4) followed by the same letter are not significantly different ($P \leq 0.05$).

Table 5. Carbon and nitrogen content, and isotopic (^{15}N & ^{13}C) composition of the below-ground (root) biomass.

Crop	BG[b]						C:N
	Biomass (g m^{-2})	(%C)	($\delta^{13}C$)	(atom% ^{13}C)	(%N)	(atom% ^{15}N)	
Wheat	122 ab	43.9	66.2 c	1.1780 c	0.81 d	0.4993 c	54.2 a
Canola	154 a	42.7	79.8 b	1.1928 b	0.86 c	0.5515 a	49.7 ab
Flax	173 a	43.0	83.8 a	1.1972 a	0.91 b	0.5147 b	47.3 b
Pea	20.7 b	41.6	57.2 d	1.1681 d	1.91 a	0.5161 b	21.8 c

[a] Within columns, means (n = 4) followed by the same letter are not significantly different ($P \leq 0.05$).

In general, plant residue decomposition is directly determined by the 'quality' of the residue; i.e., its biochemical properties. Thus, a variety of plant chemical components have been suggested as indicators, or predictors, of residue decomposition; these include neutral detergent fiber (NDF) and acid detergent fiber (ADF). Neutral detergent fiber (NDF) provides a measure of the structural components of the plant—specifically cell wall components such as hemicellulose, cellulose, lignocellulose, and lignin. Acid detergent

fiber (ADF) measures a subset of the NDF including cellulose, lignocellulose, and lignin; as such, the difference between the two provides a measure of the hemicellulose content of the residue. Results of the fiber quality assessment of the crop residues are summarized in Table 6. Whereas differences in NDF content of the AG residues were generally quite small (<3%), there were substantial differences in the BG residues; i.e., the NDF content of the BG residues increased in the order: wheat < pea ≈ flax << canola. Differences in the ADF content of the residues was apparent in both the AG and BG residues—with the ADF content of the AG residues increasing in the order: wheat ≈ pea < flax ≈ canola, and of the BG residues increasing in the order: flax < pea < wheat ≈ canola.

Results of a more detailed assessment of fiber quality are summarized in Table 7. These assessments indicate that there are significant ($P \leq 0.05$) differences in the contents of soluble lignin, cellulose, and hemicellulose in the AG residues of the four crops; and that there are significant ($P \leq 0.05$) differences in the contents of soluble lignin and hemicellulose in the BG residues. In general, the AG residues of the oilseed (canola and flax) crops have lignin-to-hemicellulose ratios that are greater ($P \leq 0.05$) than those for the cereal (wheat) and pulse (pea) crops. Conversely, significant differences in the L:hC ratios were not detected for the BG residues.

Table 6. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) content of the above-ground residues (AGR) and below-ground residues (BGR).

Crop	Above-ground plant residue		Below-ground plant residue	
	NDF[a] (%)	ADF[a] (%)	NDF (%)	ADF (%)
Wheat	56.57	40.15	35.44	48.10
Canola	59.07	50.77	62.97	48.13
Flax	57.86	48.94	47.24	36.55
Pea	57.13	42.93	45.45	42.99

[a] NDF and ADF were determined using the method of Stewart et al. (2015).

Table 7. Structural carbohydrates and lignin content of the above-ground residues (AGR) and below-ground residues (BGR)[a].

Crop	Soluble Lignin		Insoluble Lignin		Total Lignin		Cellulose		Hemicellulose		L:hC [b]	
	AGR	BGR	AGR	BGR	AGR	BGR	AGR	BGR	AGR	BGR	AGR	BGR
Wheat	1.5 b	1.6 a	25.5	27.4	27.1	29.0	4.5 a	2.9	1.8 a	0.6 b	15.5 b	54.1
Canola	0.7 c	1.0 bc	29.1	27.0	29.8	27.9	1.2 b	2.4	0.5 b	0.7 ab	55.9 a	41.1
Flax	1.4 b	0.8 c	34.3	33.3	35.7	34.2	3.9 a	2.6	1.2 ab	0.9 a	32.7 ab	36.7
Pea	2.2 a	1.4 ab	25.7	32.3	27.8	33.7	3.1 ab	2.2	1.0 b	0.8 ab	27.7 b	42.7

[a] Within columns, means (n = 4) followed by the same letter are not significantly different ($P \leq 0.05$).
[b] Lignin-to-hemicellulose ratio. Determined using the method of Sluiter et al. (2008).

Additional biochemical analyses failed to identify significant quantities of glucosinolates or related compounds (e.g., isothiocyanates) in the oilseed residues, though this most likely reflects a lack of analytical sensitivity (Martin Reaney, personal communications, 2017). Thus, these compounds could not be ruled out as potential contributing factors to the enhanced N_2O emissions associated with decomposition of canola residues.

Phase II

Residue decomposition and N_2O production. Residue decomposition studies were conducted using the ^{13}C- and ^{15}N-enriched crop residues produced in the greenhouse. Total CO_2 production increased rapidly during the first 120 to 240 h (5 to 10 d) of the incubation, then slowed over a period of three to four weeks—without reaching a steady state (Fig. 2A). During the latter part of the incubation (i.e., between 971-h and 2050-h; data not shown), the rate of CO_2 evolution in the residue-amended microcosms was the same as that in the non-amended control microcosm, indicating that the CO_2 being produced after about 30 d was sourced entirely from the soil and not the residue. Total CO_2-C production increased in the order: flax residue < wheat residue ≈ pea residue < canola residue and there was a strong positive correlation ($r = 0.978$; $P < 0.001$) between the amount of residue-C added to the microcosms and the total amount of CO_2-C evolved during the incubation.

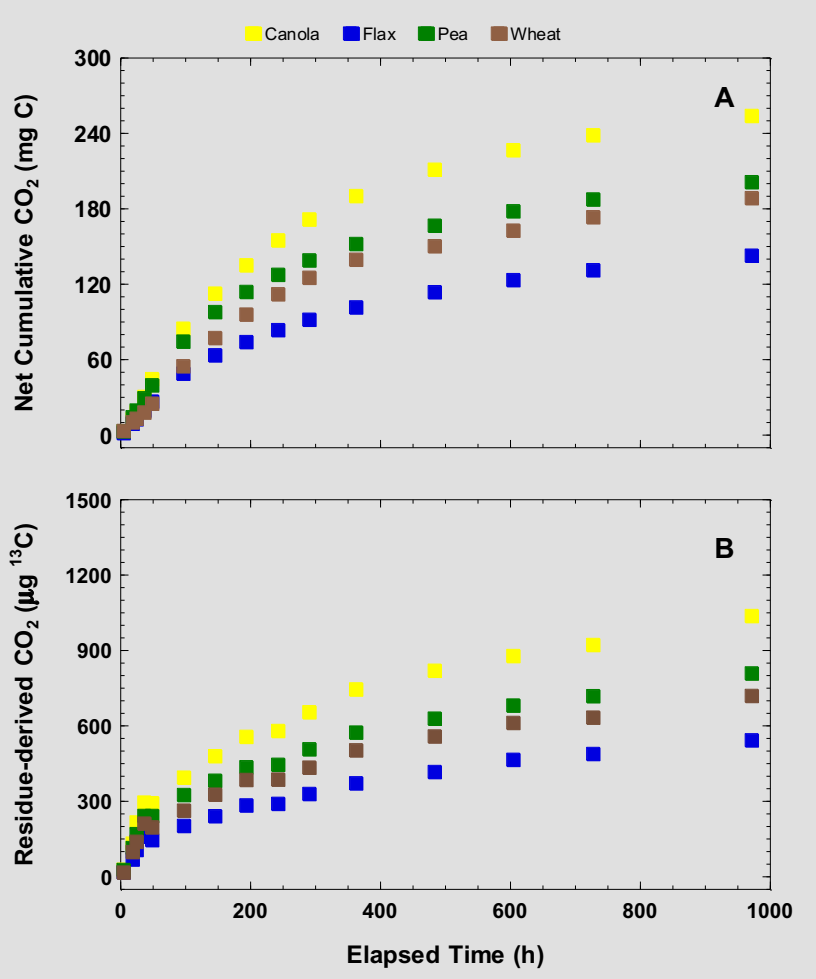

Figure 2. Total (A) and residue-derived (B) carbon dioxide (CO_2 and $^{13}CO_2$, respectively) produced during residue decomposition.

Residue-derived CO_2 (i.e., $^{13}CO_2$) production (Fig. 2B) followed a similar pattern and, again, there was a strong positive correlation (r = 0.978; $P < 0.001$ between the amount of residue-^{13}C added and the amount of $^{13}CO_2$ evolved during the incubation. In general, the crop residues contributed 16% to 19% of the total CO_2-C produced—with wheat and pea residues contributing the most and flax residue contributing the least.

In general, residue-induced CO_2 emissions (i.e., total CO_2-C – [residue-derived CO_2-C + soil-derived CO_2-C]) exceeded the residue-derived emissions (Table 8) and were greatest for the canola residue. Again, there was a strong positive correlation (r = 0.977; $P < 0.001$) between residue-induced emissions and the amount of residue-C added to the soil. These data indicate that addition of the crop residues produced a 'priming effect'; i.e., an increase in soil organic matter mineralization resulting from interactions between the microbial biomass and soil organic matter (Kuzyakov, 2010). In agricultural soils, this effect is induced by inputs of fresh organics (e.g., crop residues) and in the incubation study accounted for 35% to 48% of the total CO_2 produced.

Table 8. Cumulative CO_2-C (total and CO_2-^{13}C) produced during residue decomposition following a 7-wk incubation.

C source	Residue-C added		TCE [a,b]	SDE [a,b]	----- RDE [a,b] -----			RIE [a,b]
	(mg C)	(μg ^{13}C)	(mg C)	(mg C)	(μg ^{13}C)	(% ^{13}C)	(mg C)	(mg C)
Canola residue	466.9	5868	254.1 a	48.8	1038 a	17.7 ab	82.6 a	122.7 a
Flax residue	268.0	3360	142.9 c	48.8	543 c	16.2 b	43.4 c	50.7 c
Pea residue	342.1	4281	201.3 b	48.8	810 b	18.9 a	64.7 b	87.8 b
Wheat residue	318.5	3908	188.7 b	48.8	720 b	18.4 a	58.6 b	81.3 b

[a] Within columns, means (n = 4) followed by the same letter are not significantly different (Tukey HSD; $P \leq 0.05$).

[b] TCE = total CO_2 emissions; SDE = soil-derived CO_2 emissions; RDE = residue-derived CO_2 emissions; RIE = residue-induced CO_2 emissions.

Nitrous oxide production (both total and $^{15}N_2O$) exhibited a significant ($P \leq 0.001$) treatment effect with cumulative N_2O production in the residue-amended microcosms increasing in the order: flax < wheat ≈ pea << canola (Fig. 3). Unlike residue-C, however, there were no significant correlations between the amount of residue-N (or ^{15}N) added and the amounts of N_2O-N ($P = 0.452$) or N_2O-^{15}N ($P = 0.316$) evolved during the soil incubation. Moreover, as in our previous study (Farrell et al., 2014), residue-derived emissions (RDE) for canola (1.73%) were significantly greater than those for pea or wheat (Table 9). Unlike in our previous study, however, the RDEs for flax (0.19%) were significantly lower than those for the other crop residues. We believe that this reflects differences in the soil[8] and environmental conditions used during the incubations. Indeed, our earlier work showed that soil type had a significant effect on both the rate and magnitude of N_2O production. Perhaps more importantly, the microcosms established in the present study were maintained at about 60% WFPS as opposed to 70% WFPS in the earlier study. Thus, given that N_2O production is generally greatest at 70% WFPS, it is likely that the lower water content used in this study contributed to the lower emissions.

[8] The soil used in the present study was collected from a field site located at CSIDC in Outlook, SK; soil used in the earlier study was collected from the AAFC Research Farm in Scott, SK.

Nevertheless, the one consistent result of our research is that—under a fairly wide range of soil and environmental conditions—N_2O emissions associated with canola residue are considerably greater than those associated with wheat and pea residues.

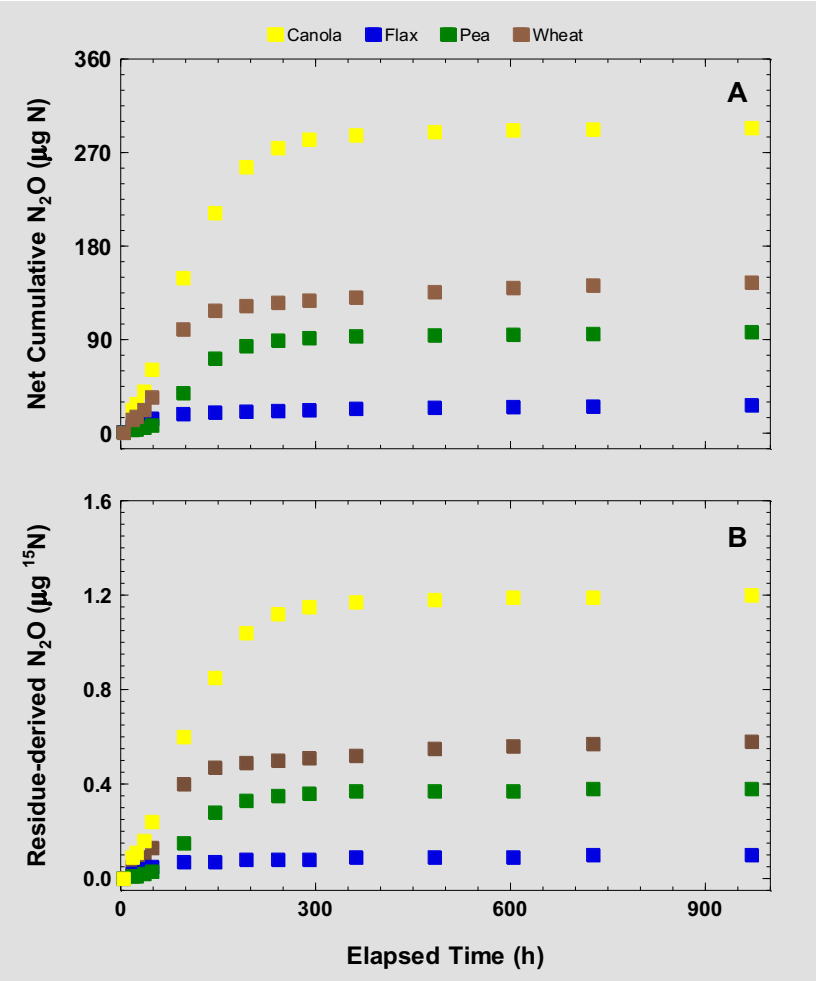

Figure 3. Total (A) and residue-derived (B) nitrous oxide (N_2O and $^{15}N_2O$, respectively) produced during residue decomposition.

In general, residue-induced N_2O emissions (i.e., total N_2O-N – [residue-derived N_2O-N + soil-derived N_2O-N]) were lower than residue-derived emissions (Table 9) and, again, there was no significant correlation ($P = 0.459$) between residue-induced emissions and the amount of residue-N added to the soil. Together, our results indicate that canola residues contribute to higher than expected N_2O emissions, not because of the added N in the residues but, more likely, because of an effect of bioactive chemicals released during residue decomposition on the soil microbial communities. For example, glucosinolates and their breakdown products—such as isothiocyanates—have been shown to have a negative effect on the structure and activity of soil bacterial communities responsible for nitrification (Bending & Lincoln, 2000; Brown & Morra, 2009). And while there is some evidence that these compounds can reduce (at least temporarily) the N_2O emissions associated with

nitrification (Balvert et al., 2017). At the same time, however, incorporation of Brassica tissues into the soil resulted in an increase in total N_2O emissions (Balvert et al., 2018), suggesting that there is a trade-off between nitrification inhibition and (possibly) denitrification-induced emissions. Moreover, given that residue quality can preselect the soil microbial community (Zafar Amin et al., 2014)—which we suspect is likely responsible for the increase in N_2O emissions observed during the decomposition of canola residues—soil samples collected at four time points during the incubation study (i.e., at the start of the incubation, during the period of rapid N_2O evolution, during the slow-down [transition] period, and after the N_2O production reached its plateau) were analyzed using quantitative PCR analysis of the genes involved in the nitrification and denitrification pathways.

Table 9. Cumulative N_2O-N (total and N_2O-^{15}N) produced during residue decomposition following a 7-wk incubation.

N source	Residue-N added		TNE [a,b]	SDE [a,b]	----- RDE [a,b] -----			RIE [a,b]
	(mg N)	(µg ^{15}N)	(µg N)	(µg N)	(µg ^{15}N)	(% ^{15}N)	(µg N)	(µg N)
Canola residue	11.336	69.77	295.4 a	1.88	1.21 a	1.73 a	196.1 a	97.4 a
Flax residue	9.794	55.05	28.6 c	1.88	0.11 c	0.19 c	18.6 c	8.1 c
Pea residue	9.924	55.36	148.5 b	1.88	0.60 b	1.08 b	107.2 b	39.4 b
Wheat residue	6.510	37.74	100.0 b	1.88	0.39 b	1.04 b	67.7 b	30.4 b

[a] Within columns, means (n = 4) followed by the same letter are not significantly different (Tukey HSD; $P \leq 0.05$).

[b] TNE = total N_2O emissions; SDE = soil-derived N_2O emissions; RDE = residue-derived N_2O emissions; RIE = residue-induced N_2O emissions.

Microbial community analysis. Denitrification can be evaluated using the N_2O production data and by quantifying *nir*K, *nir*S and *nos*Z functional genes (Appendix, Fig. A1). Quantifying functional genes estimates the number of organisms present in the soil that are capable of performing a conversion step in the nitrification or denitrification pathway (e.g. N_2O to N_2). Analysis of the qPCR data revealed that residue addition had no significant effect on the abundance of nitrification genes (A- and B-*amoA*) but had a significant influence on the denitrification genes (*nirS*, *nirK*, and *nosZ*) (Table 10). Indeed, Nemeth et al. (2014) used a combination of similar advanced molecular techniques and gaseous emissions measurements to link the abundance and activity of N cycling organisms to N_2O losses during the spring thaw period.

Our data suggest that the variation in N_2O production among residues was driven by differences in *nirS* and *nosZ* gene abundances. Positive correlations between *nirS* gene abundance and both total and $^{15}N_2O$ emissions in the residue-amended microcosms (Fig. 4) suggest that N_2O production was the result of an increase in the amount of N denitrified. At the same time, there were negative correlations between *nosZ* gene abundance and both total and $^{15}N_2O$ emissions in the canola-amended microcosms (Fig. 4) suggesting that the greater N_2O production in these systems was a result of a decrease in the reduction of N_2O to N_2.

Table 10. Repeated measure analysis of variance (ANOVA) of labelled residue application effect on N-cycling functional genes abundance (n = 16).

		A-*amoA*	B-*amoA*	*nirS*	*nirK*	*nosZ*	*nosZ* II
Treatment	F	0.644	1.833	7.996	6.945	24.140	3.420
	P	0.511	0.251	***0.015***	***0.038***	***0.007***	***0.044***
Incubation Time	F	27.423	77.196	635.800	92.409	145.531	106.395
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
Treatment × Time	F	1.302	0.723	0.597	1.672	3.367	1.028
	P	0.336	0.553	0.622	0.255	0.083	0.420

[a] Bolded and italicized *P*-values indicate that there was a significant effect of treatment (residue) or incubation time, or a significant treatment × time interaction, on N-cycling gene abundance.

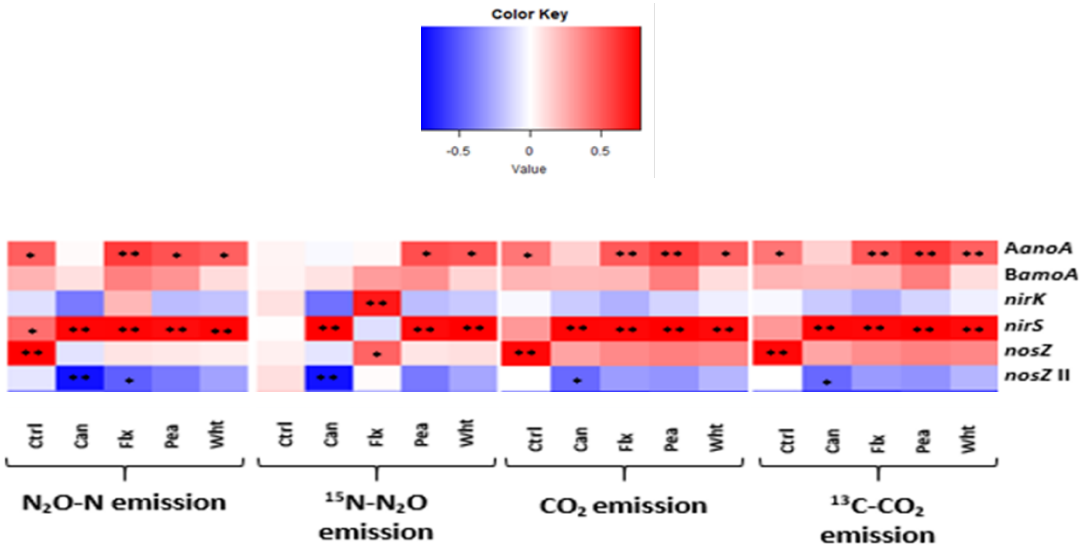

Figure 4. Spearman correlations of biogeochemical properties of incubated soil with N cycling functional genes, microbial abundance, N_2O, ^{15}N-N_2O, CO_2 and ^{13}C-CO_2 as affected by labelled residue application. **Note:** correlation coefficients range from negative (blue) to positive (red) and are indicated by colour intensity. Significant correlations are indicated by * ($P \leq 0.05$) or ** ($P \leq 0.001$). **Abbreviations:** canola (Can), flax (Flx), pea (Pea), and wheat (Wht); control (Ctrl) = unamended soil. **Functional genes:** AamoA, BamoA, nirK, nirS, nosZ, and nosZII.

Non-metric multidimensional scaling (NMDS) analysis was used to profile the microbial communities in each of the residue-amended microcosms. This analysis showed that there was a shift in microbial response over time—rather than within the residues—indicating that the microbial response was to residue decomposition and resource/substrate availability rather than residue type.

Incubation with glucosinolate or its hydrolysis product (*p-ITC*)

In general, the temporal pattern of CO_2 emissions was comparable across all treatments, though the rates of emissions were significantly affected by treatment (data not shown). Cumulative CO_2-C loss from treatments

receiving N fertilizer or fertilizer N plus *p-ITC* were not significantly different from the control (Table 11). Addition of either pea or rapeseed residue markedly increased the cumulative amount of CO_2 evolved during the incubation. Cumulative C lost as CO_2 was similar for both seed meal types (rapeseed *vs*. pea), and was not significantly influenced by the concentration of glucosinolate in the seed meal or the presence of *p-ITC*. Total CO_2-C loss represented about 0.7% of total C (i.e., soil organic C) for the control treatments and the treatment receiving an application of *p-ITC*. Emissions from treatments amended with either rapeseed or pea meal were more than 3× as high as emissions from the non-amended treatments, with the additional CO_2-C loss representing between 58% and 62% of the C added as rapeseed or pea meal. This indicates a very rapid decomposition of the added seed meal, a very strong priming effect on the existing soil organic C, or some combination the two.

Table 11. Cumulative CO_2-C produced during a 22.5-d incubation.

Treatment ID [a]	Total C (mg)	Seed meal C (mg)	Cumulative CO_2 emitted (mg C)	CO_2-C (% total C)	CO_2-C (% seed meal C)
Non-fertilized control	699.03	- - -	5.1 b	0.73	- - -
Fertilized control	699.34	- - -	5.0 b	0.72	- - -
N + *p-ITC*	699.68	0.34	5.2 b	0.74	31.5
N + L-GSL	718.11	18.77	16.8 a	2.34	62.3
N + I-GSL	718.11	18.77	16.0 a	2.23	58.5
N + H-GSL	718.11	18.77	16.2 a	2.26	59.6
N + Pea Meal	718.11	18.77	16.1 a	2.24	58.8
N + Pea Meal + *p-ITC*	718.11	18.77	16.2 a	2.26	58.4

[a] GSLs were added as freshly ground rapeseed meal.

[b] Within columns, means (n = 4) followed by the same letter are not significantly different (Tukey HSD; $P \leq 0.05$).

Unlike CO_2, N_2O emissions were substantially impacted by the addition of glucosinolate amendments to the soil. Whereas N_2O emission rates were comparable for all treatments during the first 36-h of the incubation, by DAY 4 of the incubation the rates had increased dramatically in the treatments amended with either rapeseed or pea meal (data not shown). The rate of emissions on the pea and the pea plus *p-ITC* treatments peaked at this point and then declined steadily, while the rate of emissions from the L-GSL treatment continued to increase over the next two weeks. Nitrous oxide emissions remained low on treatments receiving only fertilizer N, or fertilizer-N plus *p-ITC* and were not significantly different from the control.

Cumulative N_2O-N loss from the treatments amended with pea meal—with or without *p-ITC*—were comparable to each other and were significantly greater than the treatments without seed meal addition. They were, however, much lower than the treatments receiving glucoside-containing seed meal (Table 12). Indeed, there was a clear and substantive difference in N_2O-N loss from treatments receiving seed meal containing glucosinolates and those amended with pea meal. However, there was an inverse relationship between the

ranking of cumulative N_2O loss within the glucosinolate-containing residues and the amount of glucosinolate in the meal – i.e. total N_2O-N loss followed the order L-GSL >> I-GSL ≥ H-GSL.

Table 12. Cumulative N_2O and $^{15}N_2O$ produced during a 22.5 day incubation.

Treatment ID	N source			N_2O-N recovery			
	Seed meal	Urea		N_2O-N		N_2O-^{15}N	
	(mg N)	(mg N)	(µg ^{15}N)	(µg N)	(% added N)	(ng ^{15}N)	(% added ^{15}N)
Control (soil)	699.03	- - -	- - -	0.42 d	- - -	- - -	- - -
Soil + N	699.03	- - -	7.1	0.70 d	0.04	4.4 d	0.06
N + *p-ITC*	699.03	0.34	7.1	0.74 d	0.04	4.5 d	0.06
N + L-GSL	699.03	18.77	7.1	27.76 a	1.63	588.7 a	8.29
N + I-GSL	699.03	18.77	7.1	10.29 b	0.55	355.6 ab	5.01
N + H-GSL	699.03	18.77	7.1	8.93 b	0.45	121.6 b	1.71
N + Pea Meal	699.03	18.77	7.1	2.84 c	0.11	16.8 c	0.24
N +Pea Meal + *p-ITC*	699.03	18.77	7.1	3.29 c	0.13	20.1 c	0.28

[a] GSLs were added as freshly ground rapeseed meal.

[b] Within columns, means (n = 4) followed by the same letter are not significantly different (Tukey HSD; $P \leq 0.05$).

For all treatments receiving GSL amendments, C additions were matched and there was no significant difference in CO_2 emissions between any of the seed meal or *p-ITC* amended treatments. Furthermore, the amount of N in the L-GSL seed meal was slightly lower than the I-GSL and the H-GSL treatments, and considerably lower than that in the pea or pea plus *p-ITC* treatments, yet N_2O emissions were markedly higher on the L-GSL treatment compared to all other treatments. These observations lead us to infer that neither C or N availability—nor aeration status (CO_2 emissions were comparable, therefore O_2 consumption also would have been comparable)—were the main factors driving the differences in N_2O loss between the seed meal-amended treatments. Rather, it appears that a glucosinolate derivative was strongly impacting the N processing dynamics of the microbial community in such a way as to remarkably increase N_2O emissions. We further speculate that when present at higher concentrations, further shifts occurred that began to inhibit the production of N_2O relative to the treatments receiving lower concentrations of glucosinolate. One of the derivatives of glucosinolate-residue decomposition likely to impact microbial N dynamics is *p-ITC* (Bending & Lincoln 2000). However, in this incubation study the presence of *p-ITC* had no apparent impact on N_2O emissions, whether applied with fertilizer N or in combination with fertilizer N and pea meal. This suggests that one or more of the many other potential derivatives of glucosinolate decomposition was the factor influencing N_2O emissions in this study.

The ^{15}N-N_2O data provide further support for the inference that a glucosinolate derivative altered the microbial N processing dynamics in this incubation. The proportion of fertilizer N (source of $^{15}N_2O$) lost as N_2O was much higher (an order of magnitude or greater) in the presence of glucosinolate-containing seed meal compared to the other treatments. In the presence of glucosinolate-containing seed meal, between 1.71% and 8.29% of the fertilizer N was lost as ^{15}N-N_2O, while percent loss on other treatments ranged between 0.06% and 0.28%.

Further, the proportion of total cumulative N_2O accounted for by ^{15}N-N_2O was markedly greater when fertilizer N was in the presence of the glucosinolate-containing seed meal compared to pea meal. On all treatments receiving fertilizer N but not in the presence of glucosinolate-containing seed meal, $^{15}N_2O$-N loss consistently represented about 0.6 % of the total N_2O-N produced. This proportion increased to between 1.4% and 2.1% where fertilizer N was in the presence of glucosinolate-containing seed meal. This clearly demonstrates a strong interaction between fertilizer-N addition and seed meal containing glucosinolate. The microbial population was either relying much more heavily on the external N source (fertilizer N applied), and/or the "yield" of N_2O (N_2O produced per unit of N turned over) was much greater. This shift appears to have been modified in some fashion in the presence of high glucosinolate-containing seed meal.

Phase III (Greenhouse study)

A greenhouse study using the ^{13}C- and ^{15}N-enriched crop residues was carried out to assess the effect of residue additions (including any residue × fertilizer interaction) on N_2O emissions during wheat production. However, due to a lack of pea root material, only the canola, flax, and wheat were included in this phase of the study. In general, the CO_2 production curves (Fig. 5A) for the different crop residues followed a similar pattern; i.e., there was a short (3–4 d) lag period during which CO_2 emissions did not exceed background levels; this was followed by a 10–12 d period of rapid CO_2 production, after which CO_2 production slowed considerably until the addition of the urea fertilizer on DAY 22. Addition of the N fertilizer resulted in another increase in CO_2 production, which continued throughout the remainder of the study (i.e., until the plants were harvested on DAY 85. The $^{13}CO_2$ data (Fig. 5B) clearly show that the large bursts of CO_2 following the lag period and addition of the fertilizer N were largely a result of residue decomposition. However, the $^{13}CO_2$ emissions tended to plateau after about six to eight weeks, indicating that the emissions measured during the latter part of the study were a result of enhanced decomposition of the native soil organic matter. That is, much like in the first incubation study in Phase II, addition of the crop residues to the soils resulted in a so-called 'priming effect', or increase in soil organic matter mineralization resulting from interactions between the microbial biomass and soil organic matter. The priming effect is a natural process that is induced by pulses or continuous inputs of fresh organics (Kuzyakov, 2010)—in this case the canola, flax and wheat residues, and in the latter part of the growing season, C inputs from the growing wheat plants.

For the residue-amended soils, total CO_2-C production was greatest when the residue was wheat or canola (Table 13), but was significantly lower when the soils were amended with flax residue. As was observed in the incubation study, there was a strong positive correlation ($r = 0.998$; $P < 0.05$) between the amount of residue C (total or ^{13}C) added to the soils and the total residue-derived CO_2-C (or CO_2-^{13}C). In general, however, the proportion of residue-C emitted as CO_2 was relatively independent of the type of crop residue, ranging from about 20% to 24%. These results are in general agreement with those observed in the Phase II incubation study—though the proportion of residue-C emitted as CO_2 was somewhat greater than that found in the incubation study (see Table 8). Nevertheless, our data indicate that in terms of C cycling, the amount of CO_2 generated during crop residue decomposition was a function of the amount of residue-C added to the soil, and for canola residue was not affected by the presence of glucosinolates or their hydrolysis products.

In general, N_2O production curves (Fig. 6A) followed a similar pattern as that of the CO_2 production curves (see Fig. 5A). That is, after a short (3–4 d) lag period N_2O emissions increased for a period of 10–12 d, after which it slowed until the addition of the urea fertilizer on DAY 22. Application of the N fertilizer resulted in another increase in N_2O production, which continued for about 7–10 days before reaching a plateau at around

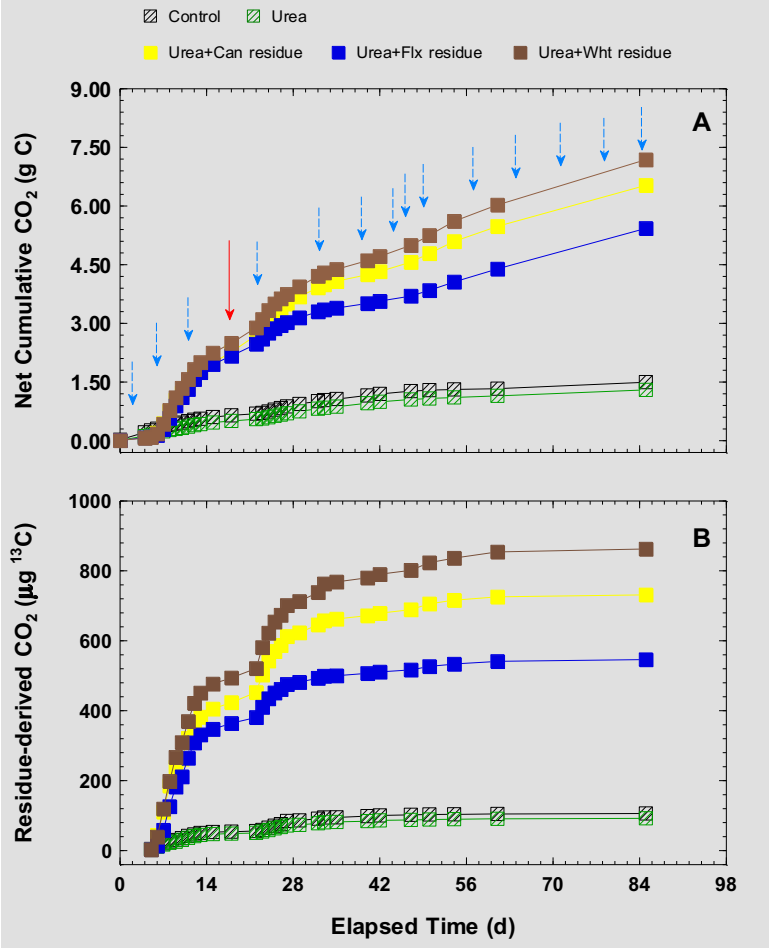

Figure 5. Total (A) and residue-derived (B) carbon dioxide (CO_2 and $^{13}CO_2$, respectively) produced during a 12-wk greenhouse study. The soils were amended with ^{13}C- and ^{15}N-labeled crop residues and non-labeled (natural abundance) urea fertilizer (equivalent to 50 kg N ha^{-1}). Note: the dashed blue arrows denote days on which the plants were watered; the solid red arrow denotes the addition of the urea fertilizer. (Can = canola; Flx = flax; and Wht = wheat.

Table 13. Cumulative CO_2 and $^{13}CO_2$ produced during a 12-wk greenhouse study.

Crop Residue	Residue-C added		TCE [a,b]	SDE [a,b]		RDE [a,b]			RIE [a,b]
	(g C)	(mg ^{13}C)	(g C)	(g C)	(mg ^{13}C)	(mg ^{13}C)	(% ^{13}C)	(g C)	(g C)
Canola	18.49	232.2	6.532 a	1.298	15.78	50.16 b	21.6	3.994 b	1.240 a
Flax	13.37	166.2	5.432 b	1.298	15.78	40.12 c	24.1	3.227 c	0.907 b
Wheat	22.42	272.2	7.186 a	1.298	15.78	56.00 a	20.6	4.612 a	1.276 a

[a] Within columns, means (n = 4) followed by the same letter are not significantly different (Tukey HSD; $P \leq 0.05$).

[b] TCE = total CO_2 emissions; SDE = soil-derived CO_2 emissions; RDE = residue-derived CO_2 emissions; RIE = residue-induced CO_2 emissions.

DAY 35. However, there was also a sharp increase in N_2O emissions between DAYs 47 and 50, which presumably reflects the addition of a large amount of water required to offset drying induced by extremely high temperatures in the greenhouse. Regardless, the $^{15}N_2O$ data (Fig. 6B) show that residue-derived emissions followed the same pattern, indicating that N_2O emissions were driven largely by decomposition of the crop residues.

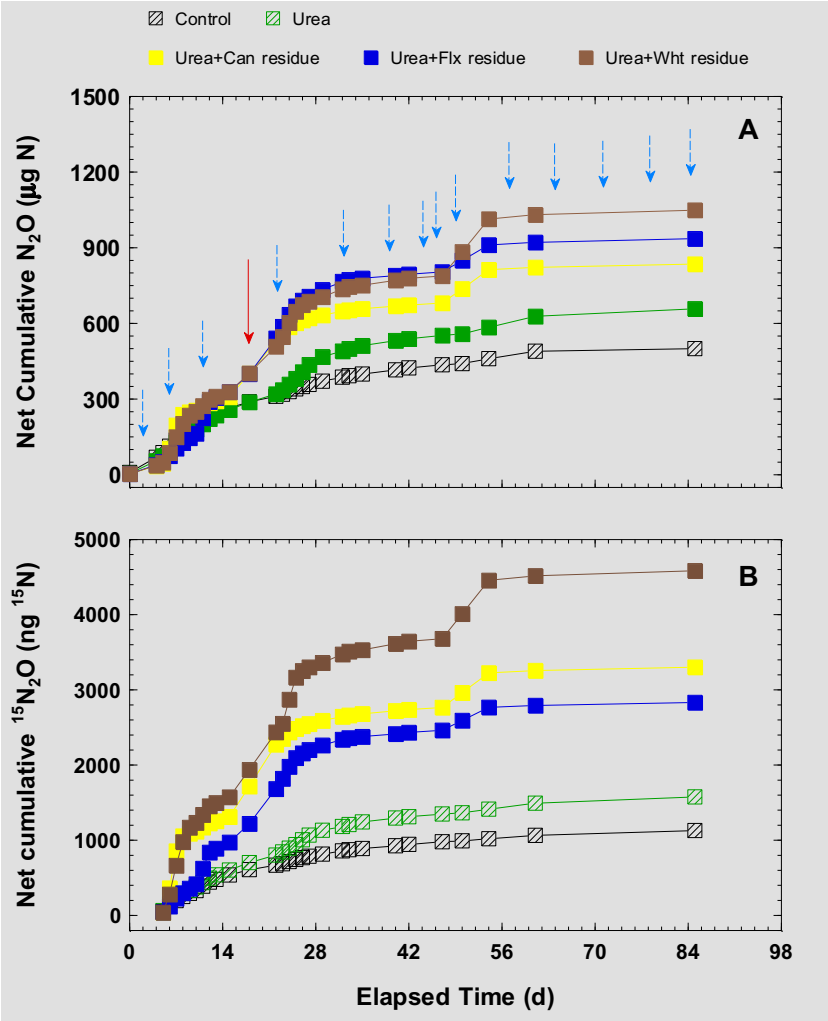

Figure 6. Total (A) and residue-derived (B) nitrous oxide (N_2O and $^{15}N_2O$, respectively) produced during a 12-wk greenhouse study. The soils were amended with ^{13}C- and ^{15}N-labeled crop residues and non-labeled (natural abundance) urea fertilizer (equivalent to 50 kg N ha^{-1}). Note: the dashed blue arrows denote days on which the plants were watered; the solid red arrow denotes the addition of the urea fertilizer. (Can = canola; Flx = flax; and Wht = wheat.

Nitrous oxide production (both total and $^{15}N_2O$) exhibited a significant ($P \leq 0.001$) treatment effect with cumulative N_2O production in the residue-amended microcosms being greater than that in the control treatment (Fig. 6 & Table 14). Recalling that the amounts of residue added were selected to yield equivalent amounts of total residue-N, it was not surprising that there was no significant correlation between the amount of residue-N

added and the amounts of N_2O-N (r = 0.163; $P = 0.896$) evolved during the greenhouse study. Neither, however, was there a significant correlation between the amount of residue-^{15}N added to the soils and the amount of N_2O-^{15}N (r = 0.158; $P = 0.867$) evolved during the 12-wk study. As with the Phase II incubation studies and our previous work (Farrell et al., 2014), these results indicate that N_2O emissions associated with crop residue decomposition are often controlled by factors other than the N content of the residues.

Table 14. Cumulative N_2O and $^{15}N_2O$ produced during a 12-wk greenhouse study.

Crop Residue	Residue-N added		Urea	TNE [a,b]	SDE [a,b]	RDE [a,b]			RIE [a,b]
	(mg N)	(mg ^{15}N)	(mg N)	(mg N)	(mg N)	(μg ^{13}N)	(% ^{15}N)	(mg N)	(mg N)
Canola	445.7	2.734	91.5	0.835 a	0.442	1.726 b	0.063 b	0.281 b	0.112 b
Flax	446.9	2.485	91.5	0.936 a	0.442	1.257 c	0.051 b	0.226 c	0.268 a
Wheat	446.2	2.500	91.5	1.049 a	0.442	3.010 a	0.120 a	0.537 a	0.070 b

[a] Within columns, means (n = 4) followed by the same letter are not significantly different (Tukey HSD; $P \leq 0.05$).

[b] TNE = total N_2O emissions; SDE = soil-derived N_2O emissions; RDE = residue-derived N_2O emissions; RIE = residue-induced N_2O emissions.

As a percentage of the residue-N applied, RDEs were much lower than those measured in the Phase II incubation study (see Table 9), which is likely a result of the different conditions under which the two experiments were conducted. For example, in the incubation study, the soil moisture content was maintained at a relatively constant level (i.e., 55% to 60% WFPS), whereas in the greenhouse study the soil water content varied over a much larger range (i.e., 40 to 70% WFPS). In addition, plant competition for available N in the greenhouse study likely reduced the amount of N available to the microbial denitrifier community. However, we did note a significant positive correlation (r = 0.998; $P = 0.027$) between N_2O and CO_2 production, suggesting a link between overall microbial activity and N_2O production. Indeed, the fact that RDEs of both N_2O and CO_2 were greatest for the wheat residue suggests that it was more easily degraded, thereby releasing more available N into the system where it could be converted into N_2O. This is supported by the residue quality assessment (see Tables 6 & 7) which found that the wheat had a relatively low content of lignin (both total and soluble) and neutral detergent fiber (NDF), both of which have been linked to enhanced degradability of crop residues (Badagliacca et al., 2017).

Assuming that the relationships between N_2O production and denitrifier gene abundances observed in the Phase II microcosm study hold true for the greenhouse study, it seems likely that the balance between N_2O production and reduction was shifted such that lower N_2O production in the canola-amended mesocosms was a result of an increase in the reduction of N_2O to N_2.

7. Conclusions and Recommendations:

The overall study consisted of a series of bench- or greenhouse-scale studies undertaken to test the factors thought to contribute to N_2O emissions from canola, flax, pea, and wheat residues. Plant materials (residues) dually labeled with ^{15}N and ^{13}C were produced during Phase I of the study so that we could track residue-derived N and C during subsequent experiments. In the first experiment, soil from a canola field was amended with

crop residues that had been finely ground to eliminate particle size effects, and then incubated at room temperature and a soil water content that equally promoted nitrification and denitrification (i.e., 60% WFPS). Whereas residue-derived CO_2 emissions were directly related to the amount of residue-C added to the microcosms, there was no significant correlation between the amount of residue-N added and residue-derived N_2O. Using a combination of advanced molecular techniques and gaseous emissions measurements we found that residue addition had no significant effect on the abundance of nitrification genes (A- and B-*amoA*), but had a significant influence on the denitrification genes (primarily *nirS* and *nosZ*). Positive correlations between *nirS* gene abundance and N_2O emissions suggest that soil incorporation of crop residues promotes N_2O production. At the same time, however, there was a negative correlation between N_2O emissions and *nosZ* gene abundance—the gene responsible for the production of nitrous oxide reductase and, in turn, the reduction of N_2O to N_2—in the canola-amended microcosms, from which we can infer that the greater N_2O production in soils amended with canola residue was a result of an increase in the 'yield' of N_2O (i.e., N_2O produced per unit of N turned over).

The second experiment involved spiking fertilized soil with freshly ground seed meal that either did (rapeseed meal) or did not (pea meal) contain glucosinolates. Results demonstrated that one or more glucosinolate derivatives strongly impacted the N processing dynamics in such a way as to produce a dramatic increase in N_2O emissions. Moreover, there was a strong interaction between fertilizer-N addition and the incorporation of glucosinolate-containing seed meal. This finding, together with those of the first experiment, strongly suggest that one or more of the derivatives of glucosinolate decomposition "turns off" *nosZ* gene expression, which results in a concomitant increase in N_2O yield.

The third experiment involved a greenhouse study in which wheat was grown in soil from a field that had been in a cereal/pulse crop rotation for four years and was amended with canola, flax or wheat residues and fertilizer-N. Unlike in the unplanted microcosm experiments, total N_2O emissions in the planted mesocosms exhibited no significant treatment (residue) effect, while residue-derived emissions from the soil amended with canola residue were lower than those from the wheat-amended soil. These results likely reflect differences in the experimental conditions; e.g., the presence of plants in the soil mesocosms is expected to exert a significant influence on both N and water availability—reducing both and limiting the formation of conditions favoring denitrification (the process most influenced by glucosinolates). As a result, residue-induced N_2O emissions were more influenced by the rate of N release from residues—with wheat turning over more quickly than canola.

Taken together, results from this study show that (i) there is there is significant potential for canola residues to enhance N_2O emissions relative to those associated with wheat, flax, and pea residues; and (ii) emissions enhancement is a result of canola residues releasing bioactive compounds during their decomposition that influence denitrifier communities in the soil—effectively increasing the yield of N_2O by inhibiting its reduction to N_2.

8. Success stories/ practical implications for industry:

The carbon intensity of commodities is an important marketing consideration. This is particularly true for canola, where access to biofuel markets is often contingent upon meeting specified carbon intensity benchmarks (e.g. European Union's Renewable Energy Directive). Thus, Best Management Practices that minimize the carbon intensity of their products need to be developed and continually refined in order for growers to remain competitive in the global marketplace. Information from this project identified the factors that influence nitrous

oxide emissions during residue decomposition, and will help point the way towards potential mitigation strategies that can be incorporated into Best Management Practices. For example, it may be possible to modify stubble management of canola to minimize N_2O emissions. Likewise, the use of inhibitors that slow the transformation of organic-N to nitrate-N (e.g., a nitrification inhibitor) may improve nitrogen use efficiency and reduce emissions.

Producers can use information from this study to improve their decision making around N management in oilseed cropping systems, bolster claims of the environmental sustainability of canola, and take advantage of environmental marketing opportunities to improve on-farm profitability.

9. Patents/ IP generated/ commercialized products:

None to report

10. List technology transfer activities:

Invited presentations:

Farrell, R.E. 2018. Greenhouse gas mitigation through nitrogen fertilizer management. Independent Consulting Agrologists Network (ICAN), Feb. 6, Saskatoon, SK (Invited talk).

Contributed presentations:

Shorunke, A.T., B.L. Helgason, R.E Farrell & Dale J. Tomasiewicz. 2017. Microbial enzyme activity in irrigated canola (*Brassica napus* L.) plots receiving different nitrogen applications. *2017 International Meeting of the ASA-CSSS-SSSA*, Oct. 22–25, Tampa, FL (Poster presentation).

Shorunke, A.T. 2019. Source partitioning approach: Understanding of greater than expected N_2O emissions from soil receiving canola (*Brassica napus* L.) residue addition. *2019 Meeting of the Canadian Society of Soil Science*, Jun. 9–13, Saskatoon, SK (Oral presentation).

Extension activities & media interviews:

Fleury D. (Interview with J.D. Knight) Belowground nitrogen contributions and other benefits of pulses in rotation. *December Pulse Advisor*, December 2016.

11. List any other funding contributions or support received.

Industry funding for this project is provided by the Saskatchewan Canola Development Commission (SCDC; fund no. 2015-83).

12. Future research potential arising from project.

Results of this study confirm that there is significant potential for canola residues to enhance N_2O emissions relative to those associated with wheat, flax, and pea residues. This potential appears to be associated with the release and subsequent transformation of glucosinolates into a variety of bioactive compounds during crop

residue decomposition. However, there is considerable variability in the glucosinolate profiles of different canola varieties, which will likely affect their impact on N_2O emissions potential. Thus future research needs include studies to (i) determine varietal differences in N_2O emissions potential enhancement; (ii) field studies to determine appropriate mitigation strategies to reduce emissions associated with canola residues; e.g., the use of a nitrification inhibitor (applied either alone or as an enhanced efficiency fertilizer), or the use of a fall cover crop to reduce fall nitrate-N levels in the soil; and (iii) field-scale research to better define the emission factors associated with canola (and other oilseed) crop residues.

13. Acknowledgements.

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14. Appendices:

A1. Literature Cited

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A2. Nitrogen turnover pathways

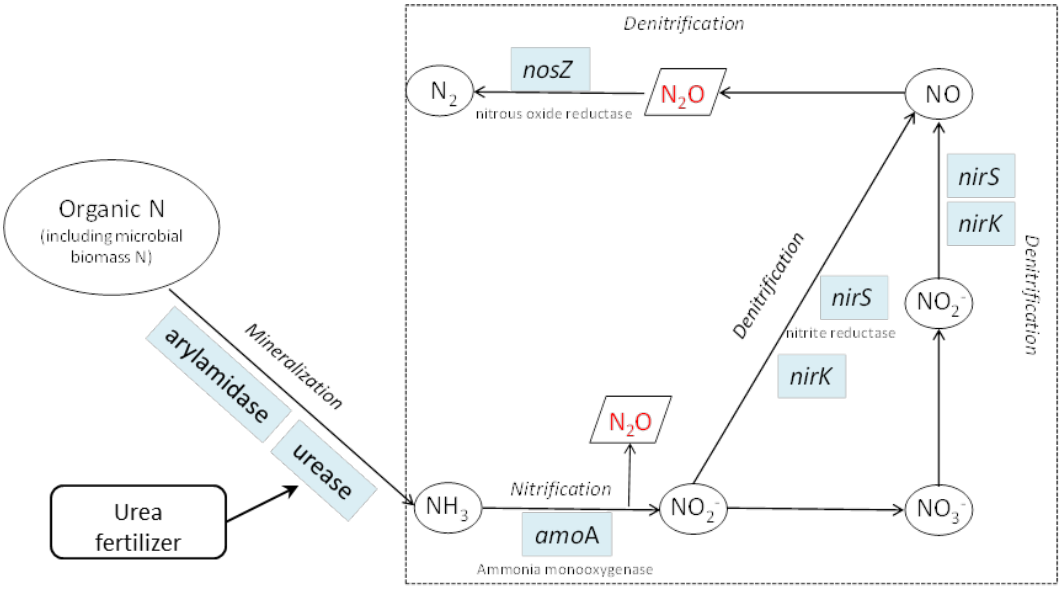

Figure A1. N turnover pathways in soil and the corresponding enzymes and genes responsible for N conversions. (Helgason et al., 2018)