Nutrient Release from Living and Terminated Cover Crops Under Variable Freeze–Thaw Cycles

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ABSTRACT
Cover crops (CC) are planted into fields during the non-growing season as a best management practice (BMP) for agronomic and environmental benefits. However, freeze–thaw cycles (FTC) may increase the availability of water extractable P (WEP) from damaged plant tissues, leading some to question their efficacy as a nutrient BMP due to their potential to release P during snowmelt. The objectives of this study were to experimentally determine the influence of: (1) FTC magnitude (4°C, −4 to 4°C, −18 to 10°C, −18 to 10°C), (2) CC species (cereal rye (Secale cereale L.), oilseed radish (Raphanus sativus L. var. oleoferus Metzg. Stokes), red clover (Trifolium pratense L.), and hairy vetch (Vicia villosa Roth]), and (3) termination using herbicide on the magnitude of WEP, NH4+ release, and NO3− release. Shoot tissue clippings underwent five FTC followed by extraction. Large magnitude FTC from −18 to 4 and −18 to 10°C (heavy frost) elevated WEP release, whereas the −4 to 4°C (light frost) treatment did not. Responses varied with plant type, where frost-intolerant species released more WEP than frost-tolerant species. In contrast, NH4+ and NO3− release did not increase following FTC. Termination elevated WEP and NH4+ release across all temperature treatments. The use of CC as a nutrient BMP should be used with caution in some regions, but in areas with mild winter climates, growing frost-tolerant species without termination may reduce the risk of P leaching from vegetation in winter and early spring.

Core Ideas
• Light frosts did not increase phosphorus release from cover crops.
• Heavy frosts released more water-extractable phosphorus than light frosts.
• Herbicide induced termination increased phosphorus and ammonium losses.
• Frost tolerant species released less phosphorus than frost-intolerant species.
• Cover crops remain a suitable management practice in temperate regions.

Elevated levels of P transported into aquatic ecosystems from agricultural watersheds have contributed to the eutrophication of surface water bodies in Canada as well as freshwater habitats around the world (Smith et al., 1998; Schindler et al., 2012; Michalak et al., 2013; Jarvie et al., 2017). In salt water environments, eutrophication has been linked to anthropogenic N inputs (Gruber and Galloway, 2008; Congreves and Van Eerd, 2015). Eutrophication is problematic as it impacts both ecosystem and human health (Anderson et al., 2002), which reduces the recreational value of lakes as well as their potential use as drinking water sources (Smith et al., 1998, 2015). Consequently, there is significant pressure to reduce the export of P and N from surrounding watersheds (International Joint Commission, 2014). Although researchers and environmental managers have attempted to manage nutrient export for decades (Sharpley et al., 1994), the occurrence of large algal blooms has increased due to a combination of climate drivers as well as the large intensity of agricultural land use in surrounding watersheds (e.g. Michalak et al., 2013; Smith et al., 2015). There is also evidence that the elevated P loads from agricultural systems may be an unintended consequence of conservation practices (Jarvie et al., 2017). Thus, an improved understanding of the efficacy of best management practices (BMPs) is needed, and potential unintended consequences must be identified and quantified.

The use of CC is a conservation practice that is growing in popularity (Wayman et al., 2016; Statistics Canada, 2017). Cover crops are plants grown by farmers for their benefits to the soil, environment, and future crop yields (Snapp et al., 2005; Blanco-Canqui et al., 2015). Cover crops have the benefit of reducing particulate P losses associated with soil erosion; however, concern has been raised about their potential to release dissolved reactive P (Tukey, 1970; Sharpley et al., 1994; Sturite et al., 2007; Liu et al., 2014). Phosphorus loss through leaching is typically minor; however, certain conditions and mechanical processes have been found to increase the concentration of nutrients in leachate (Tukey and Morgan, 1963; Bechmann et al., 2005; Lozier and Macrae, 2017; Lozier et al., 2017). Of particular importance for northern temperate regions is the effect of freezing on P release from plants,

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as damage caused by FTC can rupture cell walls, making significant amounts of nutrients available for export (Tukey and Morgan, 1963; Miller et al., 1994; Bechmann et al., 2005; Elliott, 2013). Other dissolved compounds such as N species may also be released through FTC processes (Sturte et al., 2007), although some have suggested that N may be affected to a lesser degree than P by FTC damage (Miller et al., 1994). Thus, an improved understanding of the impacts of FTC on coupled P and N release is needed to improve nutrient BMPs.

To assess the potential for winter P and N losses from CC, several studies have employed laboratory experiments. Factors that have been shown to impact the rate of P leaching from CC include the degree of FTC temperatures (Øgaard, 2015), species differences (Elliott, 2013; Liu et al., 2014), and the number of FTC (Bechmann et al., 2005). An increased number of FTC increases P release from CC, although this appears to plateau over time (Bechmann et al., 2005) and is not observed for all species (Lozier et al., 2017). The magnitude of freezing has also been found to make a difference, as plants exposed to FTC at −10°C released substantially less P than plant samples left outdoors and exposed to temperatures less than −20°C (Øgaard, 2015). With the exception of Øgaard (2015) and Lozier and Macrae (2017), most laboratory studies have used FTC temperatures such as −18 to 18°C, or −18 to 12°C (e.g. Bechmann et al., 2005; Liu et al., 2013a, 2014; Riddle and Bergstrom, 2013). Although these conditions were representative of extreme shifts in the Nordic climate (Liu et al., 2013a) or winter conditions in colder regions such as the Northern Great Plains of North America, they may not be appropriate for assessing how CC will be affected in more temperate regions where such extreme temperature fluctuations are rare. Thus, a greater understanding of the impacts of FTC magnitude on nutrient release from CC is needed.

Differences between CC species have also been found to affect potential P and N release from tissue under similar conditions (Miller et al., 1994; Elliott, 2013; Lozier and Macrae., 2017). For example, chicory (Cichorium intybus L.) and phacelia (Phacelia tanacetifolia Benth.), CC commonly used in Sweden, have minimal losses, possibly related to greater woodiness (Riddle and Bergstrom, 2013; Liu et al., 2014) whereas oilseed radish and other species susceptible to winter-killing typically leach greater quantities of P once exposed to freezing temperatures (Liu et al., 2013a, 2014; Øgaard, 2015; Lozier and Macrae, 2017).

An additional factor that may impact nutrient release from CC is termination in autumn. While some species are killed by freezing temperatures, some survive through the winter, which can be difficult to manage the following spring when fields are drying, and, waiting for termination may shorten the growing season. Consequently, some farmers choose to terminate CC prior to the arrival of winter. This is a common practice in Ontario, Canada. Termination is typically done through the application of an herbicide, but may also be done through plowing or mowing (Cherr et al., 2006; Blanco-Canqui et al., 2015). Research comparing CC to dead plant residues (left after cash crop harvest to cover the soil) has shown that living plant tissue releases significantly more dissolved P than dried residues (Elliott, 2013; Lozier et al., 2017). Lozier et al. (2017) reported that FTC did not enhance P release from red clover and suggested that this may have been due to the application of glyphosate (N-(phosphonomethyl)glycine) to the CC several days prior to the experiment. However, recently terminated CC may not desiccate immediately, and may still be potential P sources compared to dried residues that have different potential P release (Elliott, 2013). An improved understanding of the impact of termination on P release from CC is needed, as well as information on how this compares to P release from CC that are frozen while living.

Much of the evidence of winter P release from CC has been conducted in regions with harsh winters (or under harsh simulated conditions), on CC species typically grown in these regions, and on plants that are not terminated prior to freezing. The use of CC as a BMP has been widely promoted in temperate regions to reduce nutrient loading during the non-growing season as this is the period during which most P and N losses occur (Macrae et al., 2007; Congreves and Van Eerd, 2015; Blanco-Canqui et al., 2015). Some advisors have encouraged farmers to stop terminating CC or reconsider CC usage. To assess the potential for winter P and N loss from CC in the Great Lakes region of North America and to refine CC-specific nutrient BMPs, this study used a laboratory experiment (factorial design) to examine the individual and combined impacts of (i) FTC magnitude, (ii) CC species and (iii) termination on WEP, nitrate (NO_3^-) and ammonium (NH_4^+) release.

MATERIALS AND METHODS

Collection of Field Samples

Five CC species were used in this study: cereal rye, oilseed radish, red clover, oat, and hairy vetch. Samples were collected from commercial winter wheat (Triticum aestivum L.) fields in Southwestern Ontario, where wheat and CC were planted and grown according to typical grower practices. Consequently, CC were collected from different fields. Red clover was frost seeded into wheat in March. All other CC were planted after wheat harvest in late July. Oat and oilseed radish were grown together in one field and in a different field, cereal rye and hairy vetch were grown in strips. However, both fields were managed by the same grower.

Only tissue growing above ground was collected, with the exception of oilseed radish, where the tap root was analyzed separately from the shoot tissue. Cover crop samples were collected by clipping the whole shoot at the soil surface, except for oilseed radish tap roots, which were pulled out and cut at the crown separating shoot from root tissue. Living samples were collected on 21 and 22 Oct. 2015. In this manuscript, the use of the term “living” was applied to fresh vegetation samples that were collected while plants were alive, to be compared with plants that were “terminated” using herbicides. It should be noted that living vegetation samples were clipped and no longer connected to root systems at the time of extraction (and thus not actually living); however, samples were green and turgid at the time of extraction. Glyphosate was applied to fields after the first sample collection and terminated CC samples were collected approximately 2 wk later. Cereal rye and hairy vetch were sampled 13 d after application on 5 Nov. 2015, red clover after 14 d on 11 Nov. 2015, and oat and oilseed radish after 15 d on 11 Nov. 2015. All sprayed CC samples had senesced by the second collection period. Once collected, samples were bagged, stored in a cooler for transportation to the lab. A portion of each sample was...
immediately extracted (control treatment), while the bulk of the samples were separated into treatment groups. Oilseed radish roots were wet-wiped to remove all excess dirt, and all other samples were left untouched from the field. A small amount of rainfall occurred over the 2-wk period between the collection of “living” and “terminated” samples that may have leached some nutrients, and, there was a light frost. The coldest temperature recorded was ~3°C (Environment Canada, 2015a). In anticipation of this potential, a portion of the CC was left unsprayed in fields, from which a second round of unsprayed samples were collected at the same time as the terminated samples. Compared to samples collected during the first round, no significant difference was found between WEP concentrations in leachate, nor TP concentrations in plant tissues ($p > 0.05$; data not shown), indicating minimal leaching of P between the sampling periods.

### Experimental Treatments

The treatment groups were developed with a full factorial design, such that all CC tissue samples from both living and dead plants, from every species underwent all of the different FTC. The FTC regimes used were (i) a control extracted immediately on collection with no FTC, (ii) no freezing (held at 4°C), (iii) –4 to 4°C, (iv) –18 to 4°C, and (v) –18 to 10°C. Samples were kept in freezing conditions for 12 h, before moving to thawing conditions for another 12 h to complete one cycle. During the course of the FTC, samples were kept in thinly packed, large (26.8 × 27.3 cm) resealable plastic bags, such that samples were evenly exposed to FTC temperatures, and no insulating effect occurred in the middle. All samples underwent five cycles, as Bechmann et al. (2005) suggest that available P levels were not significantly increased by further freezing events. The freezing temperature of –18°C used in the current study is extreme, as is the frequency of FTC. However, this temperature and the FTC pattern used were chosen to maintain consistency with the experimental methods used in previous studies (e.g., Bechmann et al., 2005; Liu et al., 2013a, 2014; Riddle and Bergstrom, 2013) for the purpose of contrasting the impact of lighter FTC in the Great Lakes Region. After the final FTC, all samples were transferred to a 4°C refrigerator prior to being extracted for the determination of WEP, done within 24 h of the final thaw. A portion (approximately 15 g field moist) of each sample was dried at 80°C for 24 h to determine moisture content to allow nutrient contents to be expressed per dry weight mass.

### Sample Processing and Laboratory Analyses

Three replicates were sampled from each treatment group. Approximately 5 g (field moist) of shoot tissue was clipped to lengths of 4 cm or less (to fit within the 100 mL polyethylene extraction containers) after exposure to FTC. Tissue samples were batched to randomly take material from approximately 10–20 different individual plants. Oilseed radish roots were sampled as individual pieces of root, taken as ~1 cm long cylinders from the midsection of the root. Extractions for WEP and N were conducted with 50 mL of deionized water for WEP, or 2M KCl for N. Samples were shaken for 1 h to ensure thorough contact with plant material per Roberson et al. (2007). After shaking, leachate was immediately gravity filtered through Whatman No.42 filter paper until a minimum of 15 mL of filtrate accumulated.

Filtered extracts were immediately frozen and stored at –18°C until analysis (~1 mo). Several hours prior to measurements samples were fully thawed in a cool water bath (~12°C). All sample analyses were done at the Biogeochemistry Lab at the University of Waterloo. Ortho-phosphate concentrations were determined using ion chromatography (DIONEX ICS 3000 equipped with IonPac AS18 analytical column). Nitrogen analyses were conducted using colorimetric techniques (Bran Luebbe AA3, Seal Analytical, Seattle, USA, NH$_4^+$; Alkaline salicylate with hypochlorite and nitroprusside, Method No. G-102–93; NO$_3^- +$NO$_2^-$: Alkaline hydrazide sulfate with copper, Method No. G-109–94). All analysis included 5% duplicates which fell within 5% of reported concentrations. All concentrations were presented as μg nutrient g$^{-1}$ dry plant mass.

Plant samples analyzed for TP content were oven dried at 100°C, and ground before digestion. Between 0.2–0.4 g of each tissue sample underwent a sulfuric acid/lithium sulfate/selenium digestion (Parkinson and Allen, 1975). Phosphorus concentrations were analyzed with ammonium molybdate/ascorbic acid colorimetric methods (Methods G-103–93 (DRP), G-175–96 Rev. 13 (TDP, TKP) on a Bran-Luebbe Auto Analyzer III system, Seal Analytical, Mequon WI).

### Determination of Biomass Estimates and Vegetation Nutrient Pools

To convert nutrient release values from CC tissues to approximate potential field-scale P pools, estimates of dry plant biomass (kg ha$^{-1}$) were made. Several of the species of plant samples were collected from fields containing a mixture of multiple CC species planted after winter wheat, thus determining whole field biomass was not possible. Sample values for cereal rye, oat, hairy vetch, and oilseed radish (shoot and root tissue were separated; only protruding root tissue was sampled) biomass were taken from a parallel, field-scale CC experiment planted after winter wheat in August 2016, and collected from three 0.25-m$^2$ quadrats in October 2016. The estimate of biomass value of red clover was a visual estimate by former provincial cereal specialist (P. Johnson, personal communication, 2017) determined when he collected the sample in October 2015 before glyphosate application.

### Statistical Analyses

Analysis of results was done using IBM SPSS Statistics, version 21. A three-way ANOVA of CC species, FTC magnitude and life state factors was analyzed on each nutrient separately. Due to multiple significant two- and three-way interactions ($P < 0.05$), a series of two-way and one-way ANOVAs were conducted to determine how each factor influenced nutrient availability, corresponding with the study objectives. When a one-sample Kolmogorov-Smirnov test indicated a non-normal distribution, the data were transformed with the natural log. When the log-transformed data remained non-normal, the data were ranked, and analyzed via non-parametric ANOVAs. With a normal distribution, parametric ANOVAs were run, with the Bonferroni correction applied for post-hoc analysis. When significant interactions were found between two factors, the dataset was split by the levels of one factor, chosen based on the study objectives. For ranked datasets, the Scheirer-Ray-Hare test was used to examine the effects of two factors. When significant interactions existed between the two factors, the
Kruskal–Wallis test was used applied to both factors. When the Kruskal–Wallis tests indicated significant differences, the Mann–Whitney U test was applied to each category. An α value of 0.05 was applied for all statistical testing.

RESULTS

Effects of Temperature on Extractable Phosphorus and Nitrogen Concentrations in Living Vegetation

Freeze–thaw cycles increased WEP release from living vegetation; however, the magnitude of release differed with FTC magnitude and CC species (Fig. 1a). The large magnitude FTC (heavy frost) increased WEP concentrations from all species, but differences in the magnitude of increase resulted in a significant interaction between FTC magnitude and CC species ($F = 2.987, p \leq 0.001$, Table 1a). A series of one-way ANOVA tests done for individual species reported a significant effect of FTC on WEP release for all species (Table 1b). The FTC treatments readily split into two distinct categories: light frost (4°C and −4 to 4°C) and heavy frost (−18 to 4°C and −18 to 10°C) (Fig. 1a). All plant tissues frozen at −18°C exhibited increased WEP availability, with WEP concentrations increasing several orders of magnitude on average. A series of $t$ tests found no significant differences in WEP between control samples (immediately extracted), those that remained unfrozen (held at 4°C for 5 d) or those that underwent FTC at −4 to 4°C. Similarly, plants that underwent FTC at −18 to 4°C and −18 to 10°C also had no significant difference in WEP.

In living tissue, the impact of FTC magnitude on N release did not follow a similar trend compared to WEP release (Fig. 1b,c). Concentrations of extracted NH$_4$–N were highly variable with significant interaction occurring between FTC magnitude and CC species ($F = 9.445, p \leq 0.001$, Table 1a). When samples were split by species, a series of one-way ANOVA tests found significant effects of FTC for most species (Table 1b); however, no consistent trends were observed between FTC treatments (Fig. 1c). Compared to WEP and NH$_4$–N, very little NO$_3$–N (<400 μg g$^{-1}$) was extracted in any of the sample treatments (Fig. 1b). No significant effect of FTC magnitude or interaction between FTC magnitude and CC species was found (Table 1a). Oilseed radish shoot and root tissue tended to have greater NO$_3$–N release than the other CC, which was not consistent with WEP nor NH$_4$–N released from the 4°C and −4 to 4°C (light frost) treatments.

Impacts of Termination on Extractable Phosphorus and Nitrogen Concentrations

A two-way ANOVA testing the impacts of FTC magnitude and the effect of termination as factors found a significant interaction between the factors on WEP release ($F = 11.10, p \leq 0.001$, Table 1c); consequently, each level of FTC magnitude was tested separately (one-way ANOVA) to determine the impacts of termination (Table 1d). In terminated CC compared to living CC plants, WEP availability was significantly elevated in the 4°C treatment ($F = 27.23, p \leq 0.001$) and −4 to 4°C treatments ($F = 28.95, p \leq 0.001$), whereas termination had no significant effect on plants exposed to heavy frosts of −18°C (Fig. 2).
Table 1. Results of ANOVA tests on the effects of freeze–thaw cycle magnitude (FTC), cover crop species (CC), and life stage (living, terminated) on water extractable P (WEP), nitrate N, and ammonium N release. Where significant interactions were found in a two-way ANOVA the main effects were not considered.

<table>
<thead>
<tr>
<th>ANOVA test</th>
<th>Water-extractable P</th>
<th>NO₃⁻N</th>
<th>NH₄⁺-N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test Stat.</td>
<td>df</td>
<td>Sig.</td>
</tr>
<tr>
<td>(a) 2-way ANOVA testing effects of FTC × CC</td>
<td>FTC 115</td>
<td>73.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CC 37</td>
<td>19.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>FTC × CC 23</td>
<td>3.0</td>
<td>20</td>
</tr>
<tr>
<td>(b) 1-way ANOVA testing effects of CC †</td>
<td>Hairy Vetch 6</td>
<td>7.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Red Clover 23</td>
<td>35.8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cereal Rye 13</td>
<td>6.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Oat 17</td>
<td>21.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Radish Shoot 34</td>
<td>11.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Radish Root 46</td>
<td>20.9</td>
<td>4</td>
</tr>
<tr>
<td>(c) 2-way ANOVA testing effects of Life State × FTC</td>
<td>Life State 58</td>
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<td>1</td>
</tr>
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<td></td>
<td>FTC 54</td>
<td>9.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Life State × FTC 64</td>
<td>11.1</td>
<td>4</td>
</tr>
<tr>
<td>(d) 1-way ANOVA testing effects of FTC ‡</td>
<td>Control 41</td>
<td>50.3</td>
<td>1</td>
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<td></td>
<td>4°C 39</td>
<td>27.2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>–4 to 4°C 39</td>
<td>29.0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>–18 to 4°C 2</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>–18 to 10°C 0</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>(e) 1-way ANOVA testing effects of Life State × FTC</td>
<td>–4 to 4°C Live 196617</td>
<td>2.86</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>–18 to 4°C Live 39624370</td>
<td>13.98</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>–4 to 4°C Terminated 27266725</td>
<td>59.67</td>
<td>5</td>
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<tr>
<td></td>
<td>–18 to 4°C Terminated 11371777</td>
<td>1.83</td>
<td>5</td>
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</table>

† One-way ANOVA tests for individual species were only run where significant interaction was found in the 2-way ANOVA in (a)
‡ One-way ANOVA tests for individual FTC magnitudes were only run where significant interaction was found in the 2-way ANOVA in (c)

Table 1d). Once the plant tissue was severely damaged (i.e., exposure to –18°C) the maximum level of WEP was made available, and further damage, whether through frost or herbicide, did not have any additive effects. The magnitude of FTC did not impact the variability of WEP in terminated CC, but WEP in living CC was more variable with heavy frosts than light frosts (Fig. 2).

Termination significantly increased the release of NH₄⁺-N compared to non-terminated samples (F = 49.46, p ≤ 0.001; Fig. 2, Table 1c). When both living and terminated samples were pooled (in the 2-way ANOVA), significant differences in extracted NH₄⁺-N were found with the FTC treatment (Table 1c; p > 0.05); however, post-hoc analyses revealed that this was driven by a difference between the control (immediately extracted) samples and other FTC treatments and no differences were observed between the other three FTC treatments. Extractable NO₃⁻-N content was not significantly affected by termination, although heavy frost resulted in more variability in terminated samples than living samples (Fig. 2).

Of the two forms, NH₄⁺-N contributed a larger portion of total N release, with over an order of magnitude of difference between NH₄⁺-N and NO₃⁻-N. Differences in Extractable Phosphorus and Nitrogen Concentrations among Species and Life State

Nutrient release depended on CC species, their life state and magnitude of FTC (Table 1). Figures 3–5 only compare data from two FTC treatments, as no significant differences were observed between the 4°C and −4 to 4°C treatments, and the −18 to 4°C and −18 to 10°C treatments. When living (non-terminated) CC were exposed to minimal frosts no significant differences (Table 1e) were observed in WEP concentrations across species (Fig. 3); however, when plants were exposed to heavy frosts (e.g., exposure at –18 to 4°C), stratification occurred with significant differences existing among species (F = 13.98, p ≤ 0.001, Table 1c, Fig. 3b). While all living species shared the same trend of increased WEP availability under extreme freezing conditions, the range in magnitude varied greatly. In living CC, from smallest to greatest release of P, the order of species was hairy vetch, red clover, cereal ryegrass, oat, and oilseed radish (both shoot and root tissue had substantial WEP availability; Fig. 3b).

Species differences were also apparent in terminated samples. Under both temperature treatments, terminated samples of red clover, cereal ryegrass, oat, and oilseed radish shoots were all affected in a manner that was similar to living samples exposed to heavy frosts indicating that heavy frost and termination achieve the same effect in these species. However, hairy vetch and oilseed radish root differed in WEP release when terminated compared to living tissue (Fig. 3c,d). The WEP release from terminated oilseed radish root remained quite small under minimal frost, unlike its shoot tissue counterpart, irrespective of termination. The effects of heavy frost on living and terminated oilseed radish root were comparable. Alternatively, terminated hairy vetch samples had significantly greater WEP availability than living samples, even under light frosts (F = 55.63, p = 0.002). Indeed, the effect of heavy frosts did not result in further increases compared to light frosts as there was no significant difference between sprayed hairy vetch frozen at −4°C and samples frozen at −18°C.

Species differences in potential NO₃⁻-N loss were clearly divided, with oilseed radish releasing relatively larger quantities from both living and terminated samples (Fig. 4) relative to the other species. The sole exception was oat, which also released...
significantly more $\text{NO}_3^{-}-\text{N}$ when terminated ($F = 25.96$, $p = 0.007$) under both FTC regimes. No general trends were evident between the two FTC intensities comparing species differences in $\text{NH}_4^{+}-\text{N}$ loss in living tissue, except that hairy vetch had the smallest quantity of $\text{NH}_4^{+}-\text{N}$ release under both FTC treatments (Fig. 5a,b). Cereal rye (living samples) had a greater rate of $\text{NH}_4^{+}-\text{N}$ release under light frost than all species except oat, but under heavy frost, oat and oilseed radish (shoot and root) increased to similar concentrations (Fig. 5a,b). However, when terminated, oilseed radish and oat both act as greater sources of $\text{NH}_4$ than other CC and similar to how frost greatly increased the range in potential WEP availability (Fig. 5c,d).

Field Biomass and Total Phosphorus Pools

When nutrient concentrations were combined with estimated biomass data (Table 2), there was substantial variability in the magnitude of TP available among different species; however, this was the result of larger differences in biomass than the observed variability in TP content. Field scale WEP pools were estimated at to vary from 0.1 to 1.75 kg ha$^{-1}$ with light frost, and up to 13.1 kg ha$^{-1}$ under heavy frost conditions. After exposure to minimal freezing temperatures, ≤10% of TP was available as WEP across all CC species. In contrast, heavy FTC treatments (−4°C) does not appear to have been sufficient enough to cause substantial damage to plant cells, whereas the more extreme FTC temperatures (−18°C) readily damaged plant cells, leading to elevated losses of P (Tukey and Morgan, 1963; Bechmann et al., 2005; Liu et al., 2013a, 2014; Riddle and Bergstrom, 2013; Lozier et al., 2017). The temperature at which plants were thawed appeared to be less important than freezing temperature as no differences were observed between samples thawed at cooler or warmer temperatures. Indeed, in the current study, plant samples were fully thawed within 24 h at 4°C, irrespective of the freezing temperature (−4°C and −18°C).

Liu et al. (2014) indicate that the use of −18°C is an appropriate freezing event for evaluating CC usage in Nordic regions; however, it is less appropriate for simulating the climate conditions of more temperate regions such as the Great Lakes region of North America. Indeed, although temperatures of −18°C occur in the Great Lakes region, they are uncommon. Long-term (30-yr mean, 1981–2010) data for Waterloo, ON (43.45 N 80.38 W) indicate that daily low temperatures are typically on the order of −10°C or greater (Environment Canada, 2015b). Although colder temperatures (e.g., −25°C) occur, CC in the Great Lakes region of North America are typically protected from extreme weather conditions by an insulating layer of snowpack. In Waterloo, ON, snow depths of 10 cm have been observed for over a third of January and February, and depths ≥5 cm are common from the end of December to early March (Environment Canada, 2015a). Thus, the potential of CC exposure to FTC in the Great Lakes region primarily occurs prior to snow cover in December, and during the spring melt of March, during which temperatures are at or near freezing overnight, and slightly above freezing during daytime. As the results of this experiment indicated that minimal freezing (−4°C) did not have substantial effects on WEP levels, it reasonable to suggest that potential for CC to act as a P source in winter may be highly dependent on regional climate and indicates that regional CC-specific nutrient BMPs are needed. Indeed, field studies in Canada that have demonstrated substantial P loss from CC in winter have been conducted in regions with severe winter
cally associated with winter thaws and the main snowmelt pool prior to the onset of the heavy winter frost. In Ontario, gating P release from CC by enabling the plants to release the termination of CC prior to winter may be a BMP for mitigating P export. The relative ranking of hairy vetch < red clover < cereal rye < oat < oilseed radish was similar to Miller et al. (1994), who also reported that hairy vetch released less P than the two CC, oat and oilseed radish, which are readily killed by cold temperatures. However, in addition to CC event (Liu et al., 2014; Van Eebroeck et al., 2017), increasing the potential for P release from plants due to increased contact time between vegetation and runoff water (Liu et al., 2014; Lozier and Macrae, 2017). If the vegetation P pool is released prior to this occurrence, the leached P is more likely to infiltrate and be adsorbed in the soil and thus retained in the field.

In nearly all species tested, with the exception of hairy vetch, termination resulted in WEP release that was in the same range as concentrations extracted after being exposed to −18°C FTC. Therefore, herbicide induced senescence does not normally make additional P available for extraction, and appears to only make available P that would also be leached by other cellular disruption. Hairy vetch was observed to be the sole exception to this, as greater concentrations were leached after termination than in living tissues (Fig. 3c,d). In fact, after termination, hairy vetch became an equivalent P source to oat and oilseed radish. As red clover and cereal rye did not release additional P when sprayed with herbicide in the current study, they may be more suitable winter CC for farmers who require that plants are killed in autumn. However, switching from a fall herbicide application to spring termination may be more effective at retaining P with harder, frost-tolerant CC species in moderate climates.

In the case of N, the potential for terminated CC to release elevated levels of NH₄-N was evident in comparison to living crops (Fig. 2). Indeed, termination appears to be a more important factor to consider than exposure to heavy FTC (Fig. 4, 5). Thus, avoiding fall termination of a CC should be considered a BMP for minimizing N loss.

For both N and P, other management practices such as mowing or tillage may be used to terminate CC (Blanco-Canqui et al., 2015), which should be compared to herbicide application in future work. Future experiments should evaluate the practice and method of termination at a larger scale using in field studies to determine the true extent of leaching in dead CC.

**Influence of Species on Extractable Phosphorus and Nitrogen Release**

Variation between replicates in controls (plant samples extracted immediately, not exposed to FTC) indicated that some degree of natural variation exists. It seems possible that some part of the intra-species variation occurred as a result of using both stem and leaf tissues, because stems and leaves may have different P and N contents (Roberson et al., 2007; Øgaard 2015). While best effort was made to maintain a consistent ratio of stem and leaf tissues, this may remain a source of variation. Despite this, it remains important to consider both stems and leaves, as stems can make up a substantial portion of aboveground biomass, and the greater lignin content of some species may provide important resistance to FTC effects on P leaching (Liu et al., 2014) and presumably N release.

Variation in P content was observed between species, which suggests that some species may pose a greater risk for P export, and others may serve as suitable candidates for mitigating P export. The relative ranking of hairy vetch < red clover < cereal rye < oat < oilseed radish was similar to Miller et al. (1994), who also found less WEP in red clover than oilseed radish. As expected, a distinct difference emerged with species that do not winter-kill releasing less P than the two CC, oat and oilseed radish, which are readily killed by cold temperatures. However, in addition to CC...
species, other factors such as stage of development and acclimation to cooler temperatures influence susceptibility to freezing temperatures. As neither oat nor oilseed radish had large increases in P release between control (immediately extracted) samples and minimal freezing, the experimental temperature of −4°C may not have been cold enough to damage plant tissues to the point of significant P release. Despite this, quantities of P leached from heavy freezing events (−18°C) were substantially greater in oilseed radish than other species, and suggest that oilseed radish should be avoided in climates that have such extreme temperatures without insulating snow cover and where P export is of pressing concern. Additionally, the tap root of oilseed radish may pose greater risk than shoots, as (i) they are typically a large part of plant biomass; (ii) the roots may release WEP at greater levels than oilseed radish shoot tissues; and (iii) the roots are partially emergent, and are therefore not fully insulated by soils as other CC roots would be. In situations where ambient temperatures drop to around −18°C and there is no snow cover present, oilseed radish roots present tremendous risk for leaching of dissolved P to occur. Alternatively, oilseed radish can be managed by farmers to minimize tap root growth by increasing seeding density or choosing seed selections which do not produce large tap roots.

While oat does not have the same degree of risk of P loss as oilseed radish, it also appeared to be a weaker candidate species in terms of P release. Because P concentrations in leachate were on average approximately twice as large from the control (immediately extracted) to samples frozen at −4°C, there is risk that minimal to moderate freezing in field conditions may result in dissolved P leaching. Indeed, other studies have found that oat releases some P under light frosts in both field (Lozier et al., 2017) and laboratory settings (Lozier and Macrae, 2017). However, oat CC naturally senesces when exposed to freezing temperatures; which may dry out shoot tissues and prevent FTC from damaging cells, possibly reducing the potential for WEP release as seen in other species (Miller et al., 1994; Elliott, 2013). Although red clover and hairy vetch greater P release when comparing the controls (immediately extracted) to minimally frozen samples, P loss was minimal and both species seem to be suitable candidates for further field testing.

Field Biomass and Total Phosphorus Pools

Cover crop growth and estimates of field biomass were consistent with other studies in the Great Lakes region (Vyn et al., 1999; Snapp et al., 2005; Belfry et al., 2017; Coombs et al., 2017). In addition to the greater availability of WEP in oat and oilseed radish in lab extractions, the relatively larger density on field indicates greater enhanced risk for P loss from fields during the winter season. The combination of relatively large TP content but smaller WEP availability in hairy vetch highlights it as a prime candidate species for reducing P loss over red clover and cereal rye, the other two species tested that overwinter in temperate climates. The use of legumes as a CC should be done with caution. Indeed, Meisinger et al. (1991) suggested that the use of legumes could be problematic for N leaching due to their impacts on soil mineral N concentrations. However, Vyn et al. (1999) and Coombs et al. (2017) reported that legumes did not increase soil mineral N in autumn in temperate climates.

To date, most studies on winter P or N release from CC have been conducted in a laboratory setting and fewer field studies have been published. As noted earlier, laboratory investigations such as the current study represent the initial stage of P loss from a field and may not directly translate to elevated edge-of-field losses. More information is needed on the relative contributions of vegetation and soil to field-scale P loss, and, if and how this varies with CC species, topography, soil test P and/or texture. A recent study (Lozier et al., 2017) found that in soils with relatively small soil test P, concentrations of P release from red clover and oat exceeded those from soil; however, when biomass or soil bulk density were considered, the soil represented a greater P source to surface runoff than CC. Lozier et al. (2017) also demonstrated that despite the release of WEP from CC and soil in winter, very little of the released P left the field, presumably due to interactions between infiltrated water and soil. Although the capacity for soils to significantly reduce the loss of P from CC has been demonstrated in laboratory settings (Bechmann et al., 2005; Riddle and Bergstrom, 2013), few field studies exist. The determination of the ability of CC to reduce P export in a variety of settings (soil types, climate zones) will require more field studies where losses of both dissolved and sediment-bound P species are compared between fields with CC and fields that are left bare over winter.

Conclusions

Cover crops have many benefits that must be balanced with the potential impact of nutrient losses to aquatic environments. This study has shown that CC may pose a risk for greater winter P release following FTC, but not N release; however, the potential for P loss varies with both species and frost magnitude. This suggests that in regions with mild or moderate winter climates, such as the Great Lakes Region, CC should remain an agricultural BMP, whereas in colder regions, CC should be used with caution. In this study, plants exposed to a heavy frost (−18°C)
had considerably greater concentrations of extractable P compared to those that were unrefrozen (4°C) or exposed to minimal frost (−4°C). This was more apparent in frost-intolerant species such as oilseed radish, and less apparent in harder species such as hairy vetch, red clover, and cereal rye. In contrast, termination by an herbicide released both P and N from all CC, irrespective of frost magnitude. This suggests that termination should be avoided as a management practice in regions that only experience light frost, whereas fall termination may be more suitable for avoiding as a management practice in regions that only experience frost magnitude. This suggests that termination should be considered in the selection of both CC species and the use of autumn termination.

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