Reduced Potential for Nitrogen Loss in Cover Crop–Soybean Relay Systems in a Cold Climate

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Abstract

Winter cover crops might reduce nutrient loss to leaching in the Upper Midwest. New oilseed-bearing cash cover crops, such as winter camelina (Camelina sativa L.) and pennycress (Thlaspi arvense L.), may provide needed incentives. However, the abilities of these crops to sequester labile soil nutrients are unknown. To address this unknown, N in shoot biomass, plant-available N and P in soil, and NO$_3^-$–N and soluble reactive P in soil water collected from lysimeters placed at 30, 60, and 100 cm were measured in cover crop and fallow treatments established in spring wheat (Triticum aestivum L.) stubble and followed through a cover crop–soybean (Glycine max [L.] Merr.) rotation. Five no-till cover treatments (forage radish [Raphanus sativus L.], winter rye [Secale cereale L.], field pennycress, and winter camelina) were compared with two fallow treatments (chisel till and no-till). Pennycress and winter camelina were harvested at maturity after relay sowing of soybean. Winter rye and radish sequestered more N in autumn shoot biomass, ranging from 26 to 38 kg N ha$^{-1}$, but overwintering oilseeds matched or exceeded N uptake in spring, ranging 28 to 49 kg N ha$^{-1}$ before soybean planting. Nitrogen uptake was reflected by reductions in soil water NO$_3^-$–N during cover crop and intercropping phases for all cover treatments (mean = 4 mg L$^{-1}$), compared with fallow treatments (mean = 31 mg L$^{-1}$). Cash cover crops like pennycress and winter camelina provide both environmental and potential economic resources to growers. They are cash-generating crops able to sequester labile soil nutrients, which protects and promotes soil health from autumn through early summer.

Core Ideas

- Alternative, easily established winter-surviving covers are needed in the Upper Midwest.
- Cover crops sequestered N and reduced soil and soil water NO$_3^-$–N in autumn compared with fallow.
- Winter oilseed crops reduced soil water NO$_3^-$–N in autumn through soybean planting.
- Novel winter oilseeds provide environmental and economic incentives to enhance adoption.

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system, measured against tilled and no-till winter fallow, to sequester N and P and potentially mitigate nutrient loss. Cover crop treatments included autumn-planted forage radish, winter rye, field pennycress (*Thlaspi arvense* L.), and winter camelina. Plant N uptake, soil NO$_3^-$–N and available P, and soil water NO$_3^-$–N and soluble reactive P (SRP) were monitored from winter cover crop establishment through the soybean growing season over 2 yr.

**Materials and Methods**

**Study Site and Management**

This study was conducted from August 2014 through September 2016 at the USDA-ARS Swan Lake Research Farm, Morris, MN (45.68° N, 95.8° W). Research plots were established for two site-years in stubble after a wheat (*Triticum aestivum* L.) harvest on ground typically in a corn–soybean–wheat rotation. Plots were established in autumn at two separate locations, respectively, in 2014 and 2015 at the research farm. Experimental plots (3.0 × 9.1 m) were established on a Barnes loam soil (fine-loamy, mixed, superactive, frigid Calic Hapludoll) with a 2 to 5% slope, in a randomized complete block design with four replicates, in both years. Parts of the research farm are tilled drained; however, sites used for the current experiment did not have subsurface drainage tiles.

Each site-year began in autumn and continued through the harvest of the following soybean cropping phase. To enhance cover crop establishment and prevent spring wheat from reseeding, plots were sprayed with 2.3 L (1.1 kg a.i.) ha$^{-1}$ of N-(phosphonomethyl)glycine (glyphosate) at least 1 wk prior to cover crop seeding. An automated weather station on the research farm recorded all precipitation and air and soil temperatures on an hourly basis. Although the weather station monitors both rain and snow, snow accumulation on the ground surface or alteration by wind or surface vegetation was not evaluated. Additionally, soil water content was monitored with readings recorded every 4 h using volumetric water content sensors (ECH$_2$O EC-5, Decagon Devices, Meter Group) buried at depths of 30 and 60 cm within each treatment plot of one replicate block in Site-Year 1, and two replicate blocks in Site-Year 2.

In the autumn of 2014 and 2015, six treatment plots were established in wheat stubble, including a chisel-tilled fallow, a no-till fallow, and four cover crops. Planting of the four cover crops—forage radish (*Daikon*), winter rye, pennycress (*Beecher Farm*), and winter camelina (*Joelle*)—was conducted with a small no-till plot drill (Plotter’s Choice, Kasco Manufacturing) on 31 Aug. 2014 and 2 Sept. 2015 in 12 rows spaced 25 cm apart at a seeding density of 11, 76, 6.7, and 6.7 kg ha$^{-1}$ and depth of 1.3, 1.3, 0.6, and 0.6 cm, respectively. The chisel tillage (15–20 cm deep) was conducted on the same day as cover crop seeding, and the no-till fallow treatment was left undisturbed.

The following spring (20 Apr. 2015 and 15 Apr. 2016), pennycress and winter camelina treatments were broadcast fertilized with N–P–K at rates of 90–34–34 kg ha$^{-1}$ as granular urea, diammonium phosphate, and potash, respectively. Fertilization rate was not adjusted for soil nutrient status. On 30 Apr. 2015 and 22 Apr. 2016, the chisel-till treatment was disked and harrowed, and all plots were planted with glyphosate-tolerant soybean (Pioneer P09T74R2) at 445,000 plants ha$^{-1}$ with a drill seeder. Soybean row spacing was 76 cm, and four rows per plot were interseeded between cover crop rows, where present, to minimize potential crop-to-crop competition. Immediately after soybean planting, winter rye was killed with glyphosate (1 May 2015 and 22 Apr. 2016), and pennycress and winter camelina were allowed to grow to maturity. The radish cover crop was winter-killed naturally.

Oilseed harvesting occurred on 23 June 2015 and 16 June 2016 for pennycress, and on 2 July 2015 and 24 June 2015 for winter camelina. A Hege plot combine (Model 160, Hege Maschinen) was used with the header positioned above the interseeded, growing soybean plants in two 1.5-m × 10-m areas of each plot. Subsequently, glyphosate was applied as needed for control of weeds in soybean. Soybean grain was harvested at maturity in late September or early October in a 1.5-m × 10-m area in each plot with a plot combine.

**Soil Water Sample Collection**

Soil water was sampled using porous ceramic suction cup lysimeters (Soil Moisture Equipment Corporation) inserted into vertical boreholes placed in the center of the experimental plots, between crop rows, using a hydraulic soil probe. To ensure optimal soil to lysimeter contact, soil from the bottom of the core was mixed with water to form a slurry, which was placed into the borehole first followed by insertion of the lysimeters. In 2014–2015, each plot in two blocks was outfitted with two lysimeters, one at 30-cm and the other at 60-cm soil depth. In 2015–2016, each plot in three blocks was outfitted similarly, with a third lysimeter at 100-cm depth placed in the no-till fallow, winter rye, pennycress, and camelina treatments. An event delivering ≥6 mm of precipitation was established as a threshold value for priming the suction cup lysimeters. Twenty-four hours after a 6-mm event, the lysimeters were vacuum pressurized to −60 kPa with a manual hand pump. Water samples, if present, were collected using manual pumps 24 h after vacuum pressure was applied, which allowed time for water from the surrounding sphere of soil to move into the ceramic cup at the base of the lysimeter. Routine sensor checks indicated volumetric soil moisture flux with precipitation events aligned with sampling events. Due to frozen soil (5-cm soil temp < 0°C, Fig. 1), samples were not collected during winter months (December through March).

Water obtained from lysimeters was processed according to standard procedures for water preservation and analysis (USEPA, 1982; APHA, 1992) as follows. Samples were filtered with a 25-mm acrylic housed 0.45-μm pore polyethersulfone membrane syringe filter and portioned into two 20-mL subsamples for N and P analysis. All N samples were acidified with 2 μL of stock sulfuric acid per 1 mL of filtered sample and kept at 4°C until analysis. Samples for P analysis were frozen at −10°C until analysis.

**Soil and Shoot Biomass Sampling**

Soils were sampled for measurement of bulk density, soil gravimetric water content, and soil nutrient content four times each site-year: first, to establish a baseline measure, after wheat harvest...
and within days of seeding cover crops (2 Sept. 2014 and 28 Aug. 2015); second, in autumn at the same time shoot biomass was measured before the first frost (29 Oct. 2014 and 15 Oct. 2015); third, in spring after thaw and prior to soybean planting (17 Apr. 2015 and 13 Apr. 2016); and fourth, in summer after the last oilseed harvest (2 July 2015 and 24 June 2016). On all sample dates, four soil cores (3.2-cm diam.) from each plot were taken and sectioned into 0- to 30-cm and 30- to 60-cm depth increments using a truck mounted hydraulic probe (Giddings Machine Company). Two cores were used to measure bulk density and gravimetric water content, and two cores were used for nutrient analysis. Each set was composited by the two depth increments. Bulk density cores were dried at 105°C, and soil chemistry cores were dried at 37°C, followed by grinding to a fine powder with a mortar and pestle.

Cover crop shoot biomass was sampled three times each site-year to evaluate N uptake: first, in autumn to measure biomass accrual before first frost (29 Oct. 2014 and 15 Oct. 2015); second, in spring after break from winter dormancy (30 Apr. 2015 and 21 Apr. 2016); and third, at peak biomass of oilseeds before harvest (28 May 2015 and 26 May 2016). Briefly, plants were sampled along two 0.5-m lengths of crop row, dried at 45°C, ground to pass a 0.45-μm mesh, and analyzed for total N.

Further description of sampling and statistical analysis of shoot biomass data can be found in Ott (2018).

**Water, Plant, and Soil Chemical Analysis**

Water-soluble mineral N forms ($\text{NO}_3^- \text{N} + \text{NO}_2^- \text{N}$, hereafter $\text{NO}_3^- \text{N}$, and $\text{NH}_4^+ \text{N}$) were analyzed using automated Cd reduction and salicylate methods (APHA, 1992) on a continuous flow-injection analyzer (Lachat QuikChem 8500, Hach Company). Water-soluble total N was measured by electrical conductivity detection using a Lachat IL550 TOC-TN analyzer (Hach). Soluble reactive P was analyzed using automated ascorbic acid reduction method (APHA, 1992), also on the Lachat QuikChem 8500.

Dry, finely powdered soil was analyzed for N and P following standard methods of soil analysis (Sparks et al., 1996). Soil total N was analyzed by dry combustion on a LECO TrueSpec CN analyzer (LECO Corporation). Soil mineral N ($\text{NO}_3^- \text{N} + \text{NO}_2^- \text{N}$, hereafter $\text{NO}_3^- \text{N}$, and $\text{NH}_4^+ \text{N}$) extracted with 1 M KCl was analyzed by continuous flow injection, as above. Available P was extracted with 0.5 M NaHCO$_3$, following the Olsen P method (Olsen and Sommers, 1982), and analyzed by the ascorbic acid reduction method with colorimetric readings made on a Cary Bio300 spectrophotometer (Varian). Finely ground (0.45 mm) plant material was also analyzed for C and N by dry combustion and subjected to HNO$_3$, microwave digestion for inductively coupled plasma optical emission spectrometry (Vista Pro, CCD simultaneous ICP–OES spectrometer, Varian) analysis for P.

**Statistical Methods**

Data for lysimeter nutrient concentrations were separated into three biologically relevant phases: (i) a cover crop phase, from autumn seeding through spring soybean planting; (ii) an intercrop phase, from soybean planting through June or July harvest of oilseeds; and (iii) a soybean phase, from June or July oilseed harvest of winter oilseed crops through 1 September of the soybean growing season. Despite limiting sampling events to >6 mm of rainfall, suction cup lysimeters did not consistently capture soil pore water
across all replicates of each treatment or across all sample dates. Therefore, within each collection date and lysimeter depth, the measured nutrient concentration was averaged among the treatment replicates that had samples. If no sample was collected for any replicate, a missing value was entered for that sample date. Absence of a sample (i.e., a missing value) indicates only that soil moisture was insufficient for sampling and not the lack of soil water or nutrients (i.e., a zero value). For statistical analysis, each collection date was treated as a random factor within each of the three phases, and weight assigned (0–3) indicating the number of samples used to calculate that data point. This approach assured a complete and more consistent dataset that accounted for variability in the number of samples collected, and for seasonal differences such as growth phase, fertilizing, planting, and harvesting. Data for the two site-years, weighted for the number of samples for each collection date, were analyzed for fixed effects of treatment, phase, depth, and interaction effects using a generalized linear mixed model approach (PROC GLIMMIX in SAS 9.4; SAS Institute, 2014), with site-year and collection date treated as random effects. For the 2015–2016 site-year, sample collections from 100-cm-deep lysimeters were made in four treatments. Therefore, the data for this site-year were analyzed in a second mixed model, with collection date as a random factor, to test treatment, phase, depth, and interaction effects. Soil nutrient data were analyzed for each site-year separately, with sample date, treatment (including baseline), and soil depth as fixed effects, and block replication as a random effect, using the GLIMMIX procedure.

The models selected were parameterized to minimize fit-statistics criteria, including Akaike information criterion or generalized chi-square statistic. This evaluation confirmed sample date was modeled best as a random factor rather than a repeated measure, as the dataset was constructed from treatment averages within date and depth across independent lysimeters. Non-normal data were tested against a log-normal distribution, and back transformed for presentation; due to back transformation, SEM is skewed, larger on the positive side, smaller on the negative. Multiple comparisons among treatments, phases, depths, and their interactions were made using the PDIFF function (Fisher’s LSD), with a Bonferroni adjustment if the interaction effects of interest were not significant. A $P < 0.05$ was used to establish significance. Statistical comparisons of shoot biomass were made by Ott (2018); these data are provided in Supplemental Tables S1 and S2.

Water-soluble NH$_4^+$–N was occasionally, but inconsistently, measured in suction cup lysimeter water, never amounting to >5%. Due to numerous zero values, the data could not be analyzed. Soluble total N and calculated soluble organic N, which ranged from 37 to 65% of total N concentration, demonstrated the same dynamics as water-soluble NO$_3^–$–N and were not further evaluated. Plant available soil NH$_4^+$–N was also measured and statistically analyzed, but these data were not further considered because the results indicated that no differences among treatments existed, no matching analysis from lysimeters was available, and total mineral N followed the pattern of NO$_3^–$–N.

**Results**

**Precipitation, Temperatures, and Soil Water Content**

Considerable differences in total precipitation, but with some similarities in number of events over the 6-mm rainfall threshold for sampling, occurred between the two site-years of the study (Fig. 1). Total precipitation for Site-Year 1 reached 468 mm, in 74 recorded events; this year was substantially drier than the 30-yr average of 672 mm (1981–2010; NOAA-NCDCC, 2013). Total precipitation was lowest during the cover crop phase with 75 mm total over 44 events. Although four events were over the 6-mm threshold, no lysimeter leachate was collected. The spring intercropping phase received 218 mm of rain, with 11 sampling events. The summer soybean phase received 174 mm of rainfall, with four >6 mm producing leachate. The second site-year had a cumulative precipitation of 611 mm, with 208, 124, and 279 mm during the cover crop, intercrop, and soybean crop phases, respectively. Total precipitation events and the number producing leachate were 64 and 4 in the cover crop phase; 22 and 7 in the intercropping phase, and 117 and 11 during the soybean phase.

In Site-Year 1, gravimetric water content differed by sample date, soil depth, and treatment with no significant interactions; however, in Site-Year 2, differences occurred only by date and depth (Table 1). The surface 0- to 30-cm depth was significantly wetter than the 30- to 60-cm depth in autumn and spring sample dates of Site-Year 1 and Site-Year 2, respectively ($P < 0.05$). The pattern for wetter surface soils was reflected in the continuous volumetric moisture measurements in select treatment plots over both site-years (Supplemental Fig. S1). A more pronounced treatment difference in gravimetric water occurred in Site-Year 1 and was due to significantly greater soil moisture under pennycress and winter camelina than under winter rye. This pattern was evident in volumetric water content at the 60-cm depth but not at the 30-cm depth for Site-Year 1 (Supplemental Fig. S1a). In the following site-year, treatment plots with radish had substantially lower volumetric soil moisture at the 60-cm depth throughout the measurement period (Supplemental Fig. S1b).

**Lysimeter Nutrient Concentration**

Water-soluble NO$_3^–$–N, sampled by lysimeters, was highly variable and differed among treatments within cropping
phases (Fig. 2). Significant treatment, treatment × phase, and depth × phase effects occurred (P < 0.0001). Depth and other interactions were not significant. Concentrations averaged over the 30- and 60-cm depths for winter fallow systems ranged from 6.2 to 44 mg L⁻¹ over the three cropping phases. Concentrations in overwintering cover crops were <7 mg L⁻¹ during the active-growth cover crop and intercropping phases, and significantly different from both tilled and no-till fallow (Fig. 2). During the soybean phase, water-soluble NO₃⁻–N was significantly greater under the pennycress and winter camelina, compared with other treatments. This was a significant increase compared with the previous two phases, whereas NO₃⁻–N significantly decreased under the tilled and no-till fallow. Concentrations were lowest within the winter rye during cover crop phase and highest in the radish during the intercropping phase, with no differences between the other two phases. The depth by phase interaction indicated that NO₃⁻–N concentrations were significantly greater at 60 cm than at 30 cm during the intercropping and soybean phases, but not the cover crop phase, being 6.2, 8.3, and 4.4 mg L⁻¹ for 60 cm, and 4.5, 7.5, and 4.4 mg L⁻¹ at 30 cm by phase, respectively.

Soluble reactive P was not as variable, or as responsive to winter cover crop treatments as water-soluble NO₃⁻–N, ranging between 10 and 41 μg L⁻¹ (Fig. 3). Significant differences in SRP occurred among treatments (P = 0.0048), depths (P = 0.0406), and the phase × treatment interactions (P < 0.0001), but phase and other interactions were not significant. The most pronounced difference in SRP was in the pennycress treatment, which was significantly lower than all but the radish in the intercropping phase and significantly greater than all treatments during the soybean phase (Fig. 3). Over all treatments and phases, SRP was significantly greater at 30 cm than at 60 cm, averaging 20 and 17 μg L⁻¹, respectively.

For the four instrumented treatments over all phases, water-soluble NO₃⁻–N ranged from 1.3 to 20.9 mg L⁻¹ and SRP ranged from 11 to 25 μg L⁻¹ in the 100-cm-depth lysimeters (Table 2). Significant differences for water-soluble NO₃⁻–N occurred for treatment, phase, depth, and all interaction effects (P < 0.02). Significant differences in SRP occurred for treatment, depth, treatment × depth, treatment × phase, and the three-way interaction effects (P < 0.05), but phase and the depth × phase interaction were not significant. Within treatments, NO₃⁻–N concentration during the intercropping phase was significantly lower at the 100-cm depth for winter rye and winter camelina, but lower at 60 cm under pennycress (Table 2). During the soybean phase, NO₃⁻–N concentrations differed only under no-till fallow, being lowest at 30 cm. Generalizing over depths, soil water NO₃⁻–N was highest in the no-till fallow during the cover crop and intercropping phases, but higher in the pennycress and winter camelina during the soybean phase. Fewer differences in SRP were found within or among treatments. During the intercropping phase, SRP was significantly lower at 100 cm in both no-till fallow and winter camelina treatments, and during the soybean phase, it was lower at 100 cm in pennycress. Differences among treatments were limited to the 30- and 60-cm samples of the cover crop, with lower SRP more typical under winter rye for all phases (Table 2).

**Soil Nutrient Content**

Across site-years, starting baseline values for soil NO₃⁻–N were greater at the 0- to 30-cm depth at than at the 30- to 60-cm depth, with differences over time more pronounced for the 0- to 30-cm depth in Site-Year 2, and for the 30- to 60-cm depth in Site-Year 1 (Table 3). In Site-Year 1, soil NO₃⁻–N content differed by treatment (P < 0.0001), sample date (P < 0.0001), and depth (P < 0.0001), with significant interaction between treatment and sample date (P < 0.001) and treatment, sample date, and depth (P < 0.0432). In Site-Year 2, significant differences were the same (P < 0.0001, P < 0.0001, P < 0.0001, P < 0.0001, and P < 0.0293, respectively), with the exception that date by depth was also significant (P < 0.0017). Across both soil depths and site-years, soil NO₃⁻–N in tilled and no-till fallow was

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**Fig. 2.** Treatment differences in water-soluble NO₃⁻–N (mg L⁻¹) measured in lysimeter leachates collected at the 30- and 60-cm soil depths, averaged over depth and site-years by (A) cover crop phase (autumn through spring soybean planting), (B) intercropping phase (spring soybean planting through final oilseed harvest) and (C) soybean phase (after oilseed harvest through August of each growing season). Treatments are till fallow (TILL), no-till fallow (NT), winter rye (WR), pennycress (PC), winter camelina (WC), and radish (RAD). Lowercase letters indicate significant treatment differences within phases (P < 0.05). Standard error bars are shown.
other treatments in both site-years (Table 3). Radish was also
sample date.

Letters indicate significant treatment differences within phases (P < 0.05). Standard error bars are shown.

Treatments are till fallow (TILL), no-till fallow (NT), winter rye (WR),
phase (after oilseed harvest through August of each growing season).
soybean planting through final oilseed harvest), and (C) soybean
through spring soybean planting), (B) intercropping phase (spring
averaged over depth and site-years by (A) cover crop phase (autumn
in lysimeter leachates collected at the 30- and 60-cm soil depths,
ment differences in soil NO₃⁻–N at the winter oilseed harvest
at harvest. In both site-years, soil NO₃⁻–N was significantly
ing covers that ranged from 2.5 to 7.5 kg ha⁻¹ at spring sampling
fallow (20 kg ha⁻¹) than the winter rye and winter camelina
depths and sample dates was significantly greater in the tilled
winter camelina, or winter rye. In both site-years, there were no treat-
ments were taken 7 to 10 d after fertilization of the oilseeds,
additionally, the baseline (28 kg ha⁻¹) was significantly differ-
from all but the tilled fallow treatments. For both site-years
and across all sample dates, soil available P over all
depths was significantly greater in the 0- to 30-cm depth than the 30- to 60-cm depth
(Table 4). In Site-Year 1, soil available P was significantly
greater at both soil depths for the baseline and autumn sample
dates than the spring and harvest sample dates. In Site-Year 2,
the reverse occurred in the 0- to 30-cm depth and was differ-
et only between the autumn and harvest samples in the 30- to 60-cm depth.

**Shoot Biomass and Nitrogen and Phosphorus Uptake**

Over both site-years, autumn shoot biomass production for
dradish, winter rye, winter camelina, and pennycress averaged
1212 ± 124, 750 ± 90, 395 ± 56, and 180 ± 38 kg ha⁻¹,
respectively (Supplemental Table S1). The amount of N in this
biomass was 35 ± 3.8, 26 ± 2.8, 15 ± 1.7, and 6 ± 1.0 kg ha⁻¹,
respectively (Supplemental Table S2). Radish terminated over
winter and did not leave measurable dead biomass the following
spring. Spring shoot biomass of winter rye, winter camelina, and
pennycress over both site-years averaged 800 ± 283, 432 ± 153,
and 457 ± 161 kg ha⁻¹, respectively. Despite the nearly doubled
amount of winter rye shoot biomass, the amount of N in this
biomass was nearly the same for all three crops, at 35 ± 5.9, 37 ± 3.3,
and 48 ± 3.6 kg ha⁻¹, respectively. These spring measure-
ments were taken 7 to 10 d after fertilization of the oilseeds,
which might have influenced N content. Shoot biomass produc-
tion and N uptake in autumn for radish was significantly greater
in both years than for either winter camelina or pennycress; how-
ever, neither spring shoot biomass nor N uptake of winter covers
differed in either site-year (P < 0.05; Ott, 2018). Shoot P uptake
varied by cover crop, reaching 3.5 kg ha⁻¹ for radish in autumn of
Site-Year 1, which was less than the net amount for overwinter-
ing covers that ranged from 2.5 to 7.5 kg ha⁻¹ at spring sampling
over both site-years (Supplemental Table S3). At peak biomass
sampling, winter oilseeds accumulated 13 to 17 kg ha⁻¹. These
uptake values match with average concentrations of 3.3, 3.1, 3.7,
and 3.9 g kg⁻¹ for radish, winter rye, pennycress, and winter cam-
elina, respectively.
Table 2. Soil water NO$_3$−N and soluble reactive P (SRP) concentrations from four treatments instrumented with 30-, 60-, and 100-cm lysimeters over cover crop, intercrop, and soybean phases, averaged over two site-years.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Soil depth</th>
<th>No-till fallow</th>
<th>Winter rye</th>
<th>Pennycress</th>
<th>Winter camelina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cm</td>
<td>NO$_3$−N mg L$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cover crop</td>
<td>30</td>
<td>29.0 ± 15.99A†</td>
<td>0.7 ± 0.40C</td>
<td>7.3 ± 4.03B</td>
<td>1.3 ± 0.71C</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>19.6 ± 10.78A</td>
<td>1.9 ± 1.07C</td>
<td>9.5 ± 5.25AB</td>
<td>4.7 ± 2.59BC</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.4 ± 4.65A</td>
<td>1.3 ± 0.70B</td>
<td>6.8 ± 3.76A</td>
<td>1.5 ± 0.81B</td>
</tr>
<tr>
<td>Intercrop</td>
<td>30</td>
<td>40.6 ± 16.61A</td>
<td>2.5 ± 1.01B</td>
<td>5.3 ± 2.72B</td>
<td>1.3 ± 0.63Bab</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>20.8 ± 8.50A</td>
<td>0.9 ± 0.38C</td>
<td>1.3 ± 0.57Bc</td>
<td>2.8 ± 1.16B</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.9 ± 8.56A</td>
<td>0.5 ± 0.20B</td>
<td>8.0 ± 3.27Aa</td>
<td>0.8 ± 0.33Bb</td>
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<tr>
<td>Soybean</td>
<td>30</td>
<td>2.9 ± 0.99B</td>
<td>4.0 ± 1.30B</td>
<td>23.3 ± 7.52A</td>
<td>12.2 ± 3.93A</td>
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<td></td>
<td>60</td>
<td>18.0 ± 6.28AAb</td>
<td>6.9 ± 2.25B</td>
<td>22.5 ± 7.28AB</td>
<td>24.1 ± 7.79A</td>
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<tr>
<td></td>
<td>100</td>
<td>17.6 ± 5.69Ab</td>
<td>3.5 ± 1.13B</td>
<td>13.9 ± 4.51A</td>
<td>11.4 ± 3.67A</td>
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</tbody>
</table>

† Uppercase letters within a row indicate significant differences among treatments within a cropping phase and soil depth. Lowercase letters within a column indicate significant differences among depths within a treatment and cropping phase. Columns or rows lacking letters have no significant differences for the comparison.

§ Bold and italicized typeface indicates a value that is significantly different from the baseline measurement within a depth and site-year.

Table 3. Soil NO$_3$−N content ± SE by baseline and treatment within site-year and soil depths across autumn, spring, and harvest sample dates.

<table>
<thead>
<tr>
<th>Season</th>
<th>Baseline†</th>
<th>Tilled fallow</th>
<th>No-till fallow</th>
<th>Winter rye</th>
<th>Pennycress</th>
<th>Winter camelina</th>
<th>Radish</th>
<th>0- to 30-cm depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site-Year 1</td>
<td>23 ± 4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autumn</td>
<td>28 ± 7.7A†</td>
<td>24 ± 6.6Aa</td>
<td>15 ± 4.2ABa</td>
<td>13 ± 3.5Bab</td>
<td>12 ± 3.4Bb§</td>
<td>5 ± 1.4Cb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>27 ± 7.3Aa</td>
<td>36 ± 11.2Aa</td>
<td>8 ± 2.3Bb</td>
<td>12 ± 3.3Bb</td>
<td>12 ± 3.3Bb</td>
<td>20 ± 5.6Aa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harvest</td>
<td>20 ± 5.4Aa</td>
<td>23 ± 6.3Aa</td>
<td>20 ± 5.6Aa</td>
<td>23 ± 6.4Aa</td>
<td>29 ± 8.0Aa</td>
<td>21 ± 5.8Aa</td>
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<td>Site-Year 2</td>
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<tr>
<td>Autumn</td>
<td>29 ± 5.5Ab</td>
<td>22 ± 4.1AAb</td>
<td>14 ± 2.6Bb</td>
<td>16 ± 3.0Bb</td>
<td>25 ± 4.6Aab</td>
<td>6 ± 1.2Cc</td>
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<td>Spring</td>
<td>37 ± 7.0Aab</td>
<td>33 ± 6.3Aab</td>
<td>19 ± 3.6Bb</td>
<td>24 ± 4.6Aab</td>
<td>19 ± 3.6Bb</td>
<td>26 ± 4.9Abb</td>
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<tr>
<td>Harvest</td>
<td>50 ± 9.5Aa</td>
<td>50 ± 9.4Ab</td>
<td>50 ± 9.4Aa</td>
<td>38 ± 7.1Aa</td>
<td>34 ± 6.5Aa</td>
<td>52 ± 9.9Aa</td>
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<tr>
<td>Autumn</td>
<td>14 ± 3.9Ab</td>
<td>11 ± 3.0Aa</td>
<td>12 ± 3.2Aa</td>
<td>6 ± 1.7B a</td>
<td>7 ± 2.0Aa</td>
<td>4 ± 1.2Bb</td>
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<td></td>
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<tr>
<td>Spring</td>
<td>12 ± 3.4Ab</td>
<td>11 ± 3.1Aba</td>
<td>4 ± 1.2Bb</td>
<td>5 ± 1.5B a</td>
<td>6 ± 1.7B a</td>
<td>5 ± 1.5Bb</td>
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<tr>
<td>Peak</td>
<td>25 ± 6.9Aa</td>
<td>14 ± 3.9Ba</td>
<td>7 ± 2.0BCab</td>
<td>4 ± 1.2Ca</td>
<td>11 ± 3.0Ba</td>
<td>14 ± 3.9B a</td>
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<tr>
<td>Site-Year 2</td>
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<td></td>
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<tr>
<td>Autumn</td>
<td>10 ± 1.9Ab</td>
<td>8 ± 1.4Ab</td>
<td>8 ± 1.4Aa</td>
<td>8 ± 1.5Aa</td>
<td>10 ± 1.9Aa</td>
<td>2 ± 0.8C b</td>
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<tr>
<td>Spring</td>
<td>16 ± 3.0Aab</td>
<td>8 ± 1.5Bb</td>
<td>3 ± 0.6Cb</td>
<td>8 ± 1.6B a</td>
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<tr>
<td>Harvest</td>
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<td>24 ± 4.5Aa</td>
<td>11 ± 2.18Ca</td>
<td>7 ± 1.3Ca</td>
<td>6 ± 1.1Cb</td>
<td>16 ± 3.1Ab a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


‡ Uppercase letters within a row indicates a significant difference among treatments within a sample date, depth, and site-year. Lowercase letters within a column indicate a significant difference across sample dates within a treatment, depth, and site-year.

§ Bold and italicized typeface indicates a value that is significantly different from the baseline measurement within a depth and site-year.
Table 4. Soil available P content ± SE by site-year and soil depth increments across sampling dates.

<table>
<thead>
<tr>
<th>Soil depth</th>
<th>Baseline†</th>
<th>Autumn</th>
<th>Spring</th>
<th>Harvest</th>
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<tbody>
<tr>
<td>cm</td>
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<td></td>
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</tr>
<tr>
<td>Site-Year 1</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>0–30</td>
<td>41 ± 3.4as</td>
<td>35 ± 2.5a</td>
<td>14 ± 2.5b</td>
<td>18 ± 2.5b</td>
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<tr>
<td>30–60</td>
<td>14 ± 3.4bc</td>
<td>17 ± 2.5b</td>
<td>2 ± 2.5c</td>
<td>2 ± 2.5c</td>
</tr>
<tr>
<td>Site-Year 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–30</td>
<td>27 ± 3.1b</td>
<td>15 ± 2.5c</td>
<td>33 ± 2.5a</td>
<td>30 ± 2.5ab</td>
</tr>
<tr>
<td>30–60</td>
<td>4 ± 3.1de</td>
<td>1 ± 2.5e</td>
<td>5 ± 2.5de</td>
<td>5 ± 2.5d</td>
</tr>
</tbody>
</table>

† Sample dates are listed in Table 2 for each site year.
‡ Within each site-year, lowercase letters indicate differences across sample dates and depths (P < 0.05 for multiple comparisons within the sample date by depth interaction; P value adjusted for Site-Year 1).

Discussion

In the Upper Midwest, spring is considered a critical time for loss of N and P from agricultural systems due to leaching and runoff induced by snowmelt followed by heavy precipitation and low evapotranspiration demand; it is also the season when most NO₃−N enters surface or groundwater (Randall et al., 1997). This was also a time of year when soil gravimetric water content was significantly greater between the 0- to 30-cm and 30- to 60-cm depth increments in both site-years. Although soil NO₃−N analysis indicated little change in plant available N in fallow treatments from autumn to spring, the greater concern was the higher soil water NO₃−N in autumn through spring in these treatments compared with cover cropped treatments. These data indicate that measurements of soil water N, but not plant available N, are consistent with the perception that nutrient losses are more likely to occur in the spring.

Cover cropping systems can be implemented as an alternative to winter fallow systems. Living cover crops had a positive impact in reducing soil water NO₃−N concentrations compared with the tilled or no-till winter fallows, particularly from autumn through spring when NO₃−N loss was most likely. Although these observations were made on a limited dataset, they were clearly supported by the ability of cover crops to actively scavenge residual soil NO₃−N, which can vary according to soil NO₃−N level, planting time, and species (Gallagher, 1977; Delgado et al., 1999; Dabney et al., 2001). In autumn, both radish and winter rye treatments demonstrated efficiencies upward of 30 kg N ha⁻¹ at scavenging residual soil NO₃−N, evidenced also by reduction in soil water NO₃−N. On the other hand, due to cooler autumn temperatures, pennycress and winter camelina remained in a rosette stage with lower shoot biomass taking up only 5 to 16 kg N ha⁻¹ until spring (Ott, 2018), but these oilseed covers did demonstrate capacity to reduce soil water NO₃−N.

On the down side, results demonstrated that the autumn benefit of radish for sequestering soil N in living biomass was reversed by spring, as soil water NO₃−N more than doubled under the radish treatment at the intercropping phase, and spring measurements of soil NO₃−N also increased. This was likely due to decay of the radish biomass and net nitrification of N to NO₃−N, as radish does not overwinter in Minnesota. However, NO₃−N in soil and soil water under radish was at least significantly lower than the tilled fallow, if not no-till fallow, which could also indicate potential immobilization of N by microbes involved in radish biomass or wheat stubble decomposition (Kuo et al., 1996; Justes et al., 1999; Kuo and Jellum, 2002).

Under both fallow conditions, soil water NO₃−N was the same during cover crop and intercrop phases, and soil NO₃−N was also the same between autumn and spring measurements and not different from the baseline measurements given the high variability across the experimental area. Despite soybean growth and N uptake indicated by lower soil water NO₃−N during the soybean phase, soil NO₃−N content was unchanged in Site-Year 1 but increased in Site-Year 2. The overall increase in Site-Year 2 might have been due warmer soil temperatures (Fig. 1), particularly over winter, which could positively affect mineralization activity (Macduff and White 1985; Sierra, 1997; Beier et al., 2008).

The increase of soil water NO₃−N in the soybean phase in the pennycress and winter camelina treatments could be perceived as a negative aspect. However, the amount of N is half or less than that observed in soil water under tilled and no-till fallow treatments in both autumn and spring. In comparison, the reduction in soil water NO₃−N in the other four treatments was probably due to uptake by growing soybean, whereas the intercropping of oilseeds probably delayed biomass production and N uptake by soybean (Gesch et al., 2014; Berti et al., 2015). Alternatively, the increased N in soil water in the oilseed treatments might be an indication that N was being released through mineralization of oilseed shoot and root biomass. However, the fact that the oilseed covers were fertilized at the beginning of the intercropping phase cannot be disregarded. Although oilseeds accumulated a minimum of 50 kg N ha⁻¹ during this phase, fertilizer applied N not acquired by either oilseeds or intercropped soybean was a potential factor. More frequent biomass and soil measurement or tracer studies with ¹⁵N would be needed to evaluate this further.

The most notable difference in SRP was the significant increase in the pennycress treatment during the soybean phase, which followed a significantly lower SRP in the previous intercropping phase. This indicates that P might have been released through decomposition of the pennycress biomass, which is in line with the interpretation above that N was mineralized from oilseed biomass. Blackshaw et al. (2004) reported P concentrations in field pennycress biomass ranged from 1.5 to 1.7 g kg⁻¹, which is considerably more than the 0.3 to 0.5 g kg⁻¹ reported for oilseed radish (Brown et al., 2008), but only slightly more than the 1.2 to 1.4 g kg⁻¹ reported for winter rye (Karasawa and Takahashi, 2015). All of these values are below the range of P concentrations in the cover crop shoot biomass sampled in the current study. This is of interest, as a related forage radish, with similar P content, was found to increase P availability in a winter cover cropped system, though this might have been limited to the rooting zone (White and Weil, 2011). Similarly, winter rye was found to increase biomass and P uptake of a subsequent crop of soybean (Karasawa and Takahashi, 2015). Although radish appeared to decay over winter, as no substantial residue remained for spring sampling, neither plant available P nor soil water SRP changed from cover crop to intercrop phases, giving no indication of increased P availability. Winter rye sequestered some P, more in Site-Year 2, but neither plant available P nor SRP was significantly different from fallow treatments in either the intercrop or soybean phases. On the other hand, pennycress sequestered substantially more P, as

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measured in peak biomass, compared with these other two crops. However, as with all cover crop systems, but radish in particular, the potential for P loss from the system after winter thaw before first lysimeter sampling from snowmelt water (if any) moving through the soil profile is unknown.

Synchronized availability of N from mineralization of cover crop residue with demands of the following cash crop is also a concern (Dean and Weil, 2009). For radish, the increased availability of soil NO3−−N, particularly in the second site-year spring sample, was measured prior to soybean planting and continued to be reflected by soil water NO3−−N measurements during the intercropping phase. On the other hand, the overwintering covers demonstrated continued sequestration of N with lower availability of N in soil water through the intercropping phase and lack of significant change in the harvest soil sample, at least for oilseeds, despite fertilization in the spring. Although soil NO3−−N increased in winter rye at the harvest sample, significantly so in the second site-year, soil water NO3−−N content did not indicate concern for loss considering that soil N measurement required a salt solution to liberate N from the soil matrix (Mulvaney, 1996). Note that the data could not be used to specifically address nutrient loss through leaching, because nutrient concentrations could not be related back to volume within soil or moisture flux or movement through the soil column.

Potential benefits of cover crops do need to be evaluated against potential liabilities. Although autumn sequestration of N by winter rye and radish was pronounced, neither treatment indicated positive or negative effects, as soybean yields in these treatments were not statistically different than yields under fallow treatments (Ort et al., 2018). This indicates that neither sequestration, immobilization with decaying biomass, nor potential loss of nutrients from the system were of consequence in winter rye or radish. However, double-cropping oilseeds with soybean did cause a delay in soybean growth, and a reduction in soybean yields (Ort et al., 2018). On the other hand, the total production of both crops, particularly for oil content, can exceed that of monoculture soybeans, which might provide an economic incentive for adoption (Gesch et al., 2014; Berti et al., 2015; Ort et al., 2018). This combination of economic viability, environmental benefits such as floral resources for pollinators (Eberle et al., 2015; Thom et al., 2018), and the potential to reduce nutrient loss, which was established by N and P uptake and soil and soil-water dynamics in the current study, indicates that autumn-seeded, overwintering oilseed crops can provide an opportunity to increase cropping system diversity and improve economic and environmental outcomes.

Supplemental Material

Supplemental Table S1 provides the total cover crop shoot biomass measured each site-year in autumn before the first frost, spring after dormancy, and at peak shoot biomass of oilseeds. Supplemental Table S2 provides the total N taken up by cover crop shoot biomass for each site-year measurement in the autumn, spring, and at peak. Supplemental Table S3 provides data on the total P taken up by shoot biomass for each site-year. Supplemental Fig. S1 shows the soil volumetric moisture meter readings (m3 m−3) taken with ECH2O EC-5 meters (Decagon Devices, Meter Group) placed at 30- and 60-cm depths within select cover crop and fallow treatment plots each site-year.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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