Received: 3 July 2019 | Accepted: 17 December 2019

DOI: 10.1002/agj2.20119

Agronomy Journal

ARTICLE

Agronomy, Soils, & Environmental Quality

Winter canola response to soil and fertilizer nitrogen in semiarid Mediterranean conditions

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Funding information
WA Oilseeds Cropping Systems Project; USDA National Institute of Food and Agriculture, Grant/Award Number: 1014527

Abstract

In the semiarid dryland wheat (*Triticum aestivum* L.) region of the U.S. inland Pacific Northwest, winter canola (WC) (*Brassica napus* L.) is an economically viable rotation crop. Winter canola produces marketable end-products while improving soil health and disrupting pest and disease cycles. Although annual production of WC in Washington State has increased in the recent decade, little regional fertility research has been conducted. As a result, WC is commonly fertilized in a manner similar to hard red spring wheat. Compared with wheat, WC has a deep and aggressive tap root system that can grow to depths of 180 cm to reach nutrients and water. Thus, WC requires a different N management strategy than wheat. Field experiments were conducted to evaluate the influence of soil residual N and fertilizer N application rate (range, 0–240 kg N ha^{-1}) and timing (fall, spring, or split fall/spring) on WC yield and oil and protein concentrations. The study took place over a 2-yr period at seven locations across four agroecological classes. There was no yield response to N rate at six of the seven sites due to canola's high N uptake efficiency and the soils' high residual N (92–224 kg inorganic N ha^{-1}) after wheat–fallow. Increasing N rates and split or spring application resulted in lower oil/protein ratios. In addition, maximum yields correlated with total available water. Therefore, N management for WC should be based on soil test residual + mineralizable N, total available water, and end-use quality.

1 | INTRODUCTION

Winter canola or oilseed rape is well established in temperate rainfed, wheat (*Triticum aestivum* L.)-based rotations in Germany, China, the U.S. Great Plains, and eastern Canada. The primary marketable end-product is edible oil for humans, with the byproduct being sold as a protein-based meal for livestock (Assefa et al., 2018). Studies show canola also offers excellent rotational benefits through disease, weed, and pest control (Bushong, Griffith, Peeper, & Epplin, 2012; Kirkegaard, Christen, Krupinsky, & Layzell, 2008; Pan, Young, Maaz, & Huggins, 2016b). In the past decade, planted area of winter and spring canola (*Brassica napus* L.) (SC) has expanded incrementally in the wheat-dominated dryland cropping systems of the inland Pacific Northwest (iPNW) of the United States (USDA-NASS, 2019). However, scientific research and grower knowledge about best fertility management practices

Abbreviations: AEC, agroecological class; iPNW, inland Pacific Northwest of the United States; SC, spring canola; WC, winter canola; WUE, water use efficiency.

for winter canola (WC) in semiarid Mediterranean environments is limited. Winter canola has a deep and aggressive tap root system that can grow to depths of 180 cm to reach nutrients and water. Winter canola might have a higher nitrogen (N) requirement than soft white and hard red spring wheat grown in the region (Koenig, Hammac, & Pan, 2011). Thus, wheat growers must adjust their N management strategy when adapting canola to maximize agronomic and economic return on fertilizer investment and to minimize N loss to the environment (Rathke, Behrens, & Diepenbrock, 2006).

Canola N requirements are based primarily on yield goal, which is largely determined by the amount of precipitation. Although WC can exhibit 50–100% higher yield potential than SC in the iPNW (Sowers, 2018), N fertility recommendations have often not clearly distinguished between winter and spring types (Koenig et al., 2011). Spring canola N recommendations are highly variable, with total unit N requirements ranging from 4.8 to 13.5 kg N ha^{-1} per 100 kg of seed; these N requirements are supplied by a combination of (i) N in the soil profile to 90 cm, (ii) potential mineralizable N, and (iii) N applied as fertilizer (Jones & Olson-rutz, 2016; Mahler, 2005; Pan, McClellan Maaz, Hammac, McCracken, & Koenig, 2016a). The unit N requirement is the inverse of N use efficiency, which is defined as yield per unit of total N supply at optimal yield (Djaman, Bado, & Mel, 2016; Pan et al., 2016a). However, this definition of N use efficiency does not account for N contributions from soil residual inorganic N and mineralizable organic N.

Approximately 70% of precipitation in the iPNW occurs from October to March, and 25% occurs in the spring (Kruger, Allen, Abatzoglou, Rajagopalan, & Kirby, 2017). Summer months are warm and dry. Winter canola is planted in all four agroecological classes (AECs) in the Pacific Northwest: (i) annual crop (>450 mm annual precipitation), (ii) annual crop–fallow transition (300–450 mm annual precipitation), (iii) grain–fallow (<300 mm annual precipitation), and (iv) irrigated (Huggins, Rupp, Kaur, & Eigenbrode, 2014; Kruger et al., 2017; Pan et al., 2016b). The four AECs vary greatly in precipitation and in soil and air temperatures, resulting in large variability in canola yield potential (Hammac, Maaz, Koenig, Burke, & Pan, 2017; Pan et al., 2016b). Previous studies have shown that SC grown in different AECs has different responses to N fertilization. A greater unit N requirement was found in regions with low yield potential, where water stress is more likely to occur (Maaz, Pan, & Hammac, 2016; Pan et al., 2016a). Winter canola yield and quality response to N fertilization rate and timing likely differ across the variable AECs in the iPNW because much of the annual precipitation occurs during the winter and early spring.

Winter canola has three distinct phases of N use (Reese, 2015). In the fall, the crop accumulates between 25 and 30% of its N, taking up 40–150 kg N ha^{-1} (Rathke et al., 2006; Reese, 2015; Wysocki, Corp, Horneck, & Lutcher, 2007). Over winter, approximately two-thirds of this accumulated N is retained in the plant to fuel spring growth, and one-third is released back to the soil via leaf litter. Dejoux, Recous, Meynard, Trinsoutrot, and Leterme (2000) found that 50% of the N lost as leaf litter was taken back up by the plant from the time of spring growth until harvest. Upon spring green-up, the crop undergoes a period of rapid N uptake that continues through flowering. The crop then accumulates the remaining N for seed production (Wysocki et al., 2007). Timing of N applications must be managed to ensure N availability during peak uptake periods while minimizing excess N. After harvest, surplus N can remain in the soil and continue to increase through the fall due to residue mineralization, resulting in high leaching potential (Sieling & Kage, 2006).

Core Ideas

- Soil N supply in 7 site-years ranged from 92 to 224 kg N ha^{-1} after wheat–fallow.
- Winter canola maximized yield in 6 of 7 site-years without additional fertilizer N.
- Winter canola yields were correlated with total water availability.
- Increased N supply and heat stress during flowering caused decreased oil/protein ratio.
- Nitrogen fertilization should be based on soil residual N, available water, and end-use quality.

Canola yield response to applied N is strongly influenced by soil N supply (i.e., soil test residual inorganic N and N mineralization estimates) and available water. Large differences in N requirement across years and sites have been observed in SC (Pan et al., 2016a). Differences in yield potential and seasonal growth habits between SC and WC dictate that N rate and timing recommendations of WC may differ from SC in the iPNW.

High levels of N availability influence the end-use qualities of canola oil and meal by increasing seed protein concentration and decreasing seed oil concentration (Gao et al., 2010; Hocking & Stapper, 2001). Depending on market demand, oil or protein production may be more profitable and subject to premiums in any given year. For these reasons, the intended end use of canola and whether maximizing oil or protein is more favorable must also be considered when making N management decisions.

The objectives of this study were (i) to determine the influence of soil N supply and fertilizer N rate and timing effects on WC yield and seed oil and protein concentrations, (ii) to evaluate the effect of variable soil N supply on N fertilizer responses of WC in the four AECs of the iPNW, and (iii) to assess the accuracy of current regional iPNW N recommendations for producing high-quality WC in the AECs.

TABLE 1 Soil type and taxonomic classification of study sites

Site	Soil type	Taxonomic classification
Hartline, WA	Magallon sandy loam	Sandy, mixed, mesic Aridic Haploxerolls
Odessa, WA	Renslow silt loam	Coarse-silty, mixed, superactive, mesic Calciargidic Argixerolls
St. John, WA	Athena silt loam	Fine-silty, mixed, superactive, mesic Pachic Haploxerolls
Almira, WA	Bagdad silt loam	Coarse-silty, mixed, superactive, mesic Calcic Argixerolls
Echo, OR	Ritzville very fine sandy loam	Hermiston series, Coarse-silty, mixed, superactive, mesic Cumulic Haploxerolls
Endicott, WA	Hermiston silt loam	Coarse-silty, mixed, superactive, mesic Calcidic Haploxerolls
Latah, WA	Naff-Garfield complex	Fine-silty, mixed, superactive, mesic Typic Argixerolls

TABLE 2 The seven study sites by crop-year and descriptions of associated winter canola cultivar; planting and harvest dates; and soil pH, organic matter (OM), soil test P, K, and S in the upper 15 cm

Site	AEC[a]	Rotation[b]	Yield Goal	Recommended N rate[c]	Cultivar[d]	Planting date	Harvest date	Soil test[e]				
								pH	OM	P	K	S
			$kg\ ha^{-1}$	—$kg\ N\ ha^{-1}$—					%	—$mg\ kg^{-1}$—		
2016–2017												
Hartline, WA	GF	SW-F	1800	38	Claremore	27 Aug.	8 July	6.5	0.9	8	506	6
Odessa, WA	IR	WW-F	3500	187	Amanda	12 Sept.	14 July	7.2	2.0	12	220	10
St. John, WA	AFT	WW-F	3500	74	EdiMax	3 Sept.	23 July	5.1	3.2	24	694	21
2017–2018												
Almira, WA	GF	SW-F	3000	122	Claremore	27 Aug.	24 July	5.6	1.2	28	566	23
Echo, OR	IR	WW-F	3500	173	EdiMax	6 Sept.	3 July	7.4	1.0	25	485	40
Endicott, WA	AFT	WW-F	3000	98	HyClass 225W	12 Aug.	31 July	5.5	1.6	32	620	20
Latah, WA	AC	WW-F	3300	40	HyClass 225W	15 Sept.	6 Aug.	5.0	2.5	30	472	22

[a]AC, annual crop; AEC, agroecological class; AFT, annual crop–fallow transition; GF, grain–fallow; IR, irrigated.

[b]F, fallow; SW, spring wheat; WW, winter wheat.

[c]Based on unit N requirement of 7 kg N 100 kg seed yield^{-1} (Koenig et al., 2011).

[d]Claremore, Amanda, and HyClass are open pollinated varieties; EdiMax is a hybrid variety.

[e]pH: 1:1 soil/H_2O method; OM = soil organic matter, Walkley-Black method; P and K = phosphorus and potassium, Olsen method; S = sulfur, $Ca(H_2PO_4)_2$*H_2O extraction method (Miller, Gavalk, & Horneck, 2013).

2 | MATERIALS AND METHODS

2.1 | Site description

Field experiments were conducted over two crop years, from 2016 to 2018, in seven commercial WC fields across Washington and Oregon, which represented all four AECs of the iPNW. Sites differed widely in precipitation and soil type (Table 1). Specific site locations and associated soil conditions, crop rotations, WC cultivars, and planting and harvest dates are summarized in Table 2. Winter canola cultivar, seeding rate, row spacing, planting date, and other cultural practices were determined by each collaborating grower. Typical seeding rates were 4.5 kg ha^{-1}, and typical row spacing was 30–41 cm.

Climate data were collected from the online climate datasets provided by National Oceanic and Atmospheric Administration's National Centers for Environmental Information (https://www.ncdc.noaa.gov) and the U.S. Bureau of Reclamation's AgriMet (https://www.usbr.gov/pn/agrimet).

2.2 | Experimental design

We used a split-plot experimental design with either three or four replications, depending on suitable available land area at each site. Main plot treatments were three N application timings: fall, spring, and split (50% of total N rate applied in fall and 50% applied in spring). In the 2016–2017 crop year, subplot (9 m by 12 m) treatments consisted of a control and three N rate treatments: (i) the recommended rate by Washington State University Extension, (ii) 50% higher than the recommended rate, and (iii) 50% lower than the recommended rate. The Extension-recommended rates were calculated based on yield goal method adjusted by precipitation and pre-plant soil N at each site (Koenig et al., 2011). Because we observed limited yield response to N fertilization in the 2016–2017 crop year, we increased fertilization rates in the 2017–2018 crop year. In the 2017–2018 crop year, subplot (9 m by 12 m) treatments consisted of a control and five N rates equal to 45, 90, 135, 180, and 224 kg N ha^{-1}. All treatments were replicated four times except at the Latah and Echo sites, which were replicated three times due to limitations caused by size, slope, streams, and/or roads. All sites were established on an area of field with minimal slope. Either granular urea or liquid urea ammonium nitrate fertilizer was applied in the fall; only granular urea was applied in the spring.

In both crop years, N rate treatments were applied with the three timing treatments of fall, spring, and split applications. Fall applications occurred between 3 and 5 wk after planting. Spring applications occurred at spring green up, between February and April, depending on the site. Sulfur rates of 56 kg ha^{-1} ammonium sulfate (20-0-0-24) were applied in the fall at each site. Urea and ammonium sulfate were applied by handheld broadcast spreader. The urea ammonium nitrate was applied with a tractor-mounted spreader. Soil test P levels were greater than the agronomic threshold of 13 kg ha^{-1} in all sites but Hartline (Koenig et al., 2011). Farmers typically apply 11–44 kg P_2O_5 ha^{-1} with seed or banded at planting for WC.

2.3 | Soil and plant sampling and analysis

Soil samples were collected after planting and before fall fertilization and again in the spring between February and March before spring fertilization. In the fall post-plant sampling, one sample consisting of three subsamples were taken from each main plot for baseline soil testing. A soil sample was collected from each subplot in the spring and in the fall after harvest. We used a Giddings probe mounted on a pickup truck to obtain all soil samples except for spring sampling at Latah and Almira. At these two sites, samples were obtained with a hand probe because excess wetness prevented access with the pickup truck. Samples were collected to a depth of 180 cm and divided into 30-cm segments to determine total root zone available N and water.

Soil samples were stored at −15°C until analysis. A portion of the surface soil samples to 30 cm, divided into 15-cm segments, was collected at fall post-plant and sent to a commercial soil testing laboratory (Best Test Analytical Services) for general fertility analysis (Table 2). All soil samples through 180 cm at 30-cm increments were tested for moisture content and mineral N, including ammonium N (NH_4^+–N) and nitrate N (NO_3^-–N) content extracted in 1 M KCl (Gavlak, Horneck, & Miller, 2005). Soil mineral N was determined using the gas diffusion and zinc reduction–electrical conductivity detection method (TL2800 Dual Channel Analyzer, Timberline Instruments).

Winter canola was harvested using a plot combine with 1.5 m header width (Wintersteiger Nursery Master). An area of 5 × 1.5 m was harvested in each subplot. Seed was air dried for >48 h to consistent weights in a greenhouse that reached >50°C during the day. All seed was thoroughly cleaned using a 2-mm sieve and a blower and then weighed to determine yield. Seed oil and protein concentrations were analyzed using near infrared spectrometry (XDS Rapid Content Analyzer with a type XM-1000 Monochromator, FOSS Analytical) (Rathke et al., 2006). In each plot, a separate 1-m^2 section of total plant biomass was cut and bagged, dried for >48 h in the same greenhouse, and weighed to estimate total biomass yield at harvest.

2.4 | Data analysis

Available soil water was estimated as the difference between total volumetric water content at field capacity and water content at the permanent wilting point. Wilting point was determined for each site by applying equations from the Saxton-Rawls method (Saxton & Rawls, 2006) to soil texture data obtained from the NRCS web soil survey (https://websoilsurvey.sc.egov.usda.gov/). If calculated water content was below the determined permanent wilting point, we assumed soil available water was zero. We used the following equations:

$$\text{gravimetric water content}\left(\frac{\text{g water}}{\text{g soil}}\right)=\frac{(\text{soil wet weight})-(\text{soil oven-dry weight})}{\text{oven-dry weight}}$$

$$\text{volumetric water content}\left(\frac{\text{mm}^3\ \text{water}}{\text{mm}^3\ \text{soil}}\right)=(\text{gravimetric water content})\times(\text{soil bulk density})$$

$$\text{available soil water content}\left(\frac{\text{mm}^3\ \text{water}}{\text{mm}^3\ \text{soil}}\right)=(\text{volumetric water content})-(\text{permanent wilting point})$$

$$\text{available soil water (cm)}=(\text{available soil water content})\times(\text{length of increment; cm})$$

Total available water was calculated as available soil water at fall soil sampling + precipitation from time of fall sampling through harvest + total season irrigation, when applicable.

When water is a limiting factor and the relationship between yield and water is linear, yield potential can be predicted based on available water (Harmsen, 2000; Pan et al., 2016a). Harmsen (2000) defined the relationship between water and yield as:

$$A=\text{WUE}\times\left(\text{H}_2\text{O}_t-\text{H}_2\text{O}_0\right)$$

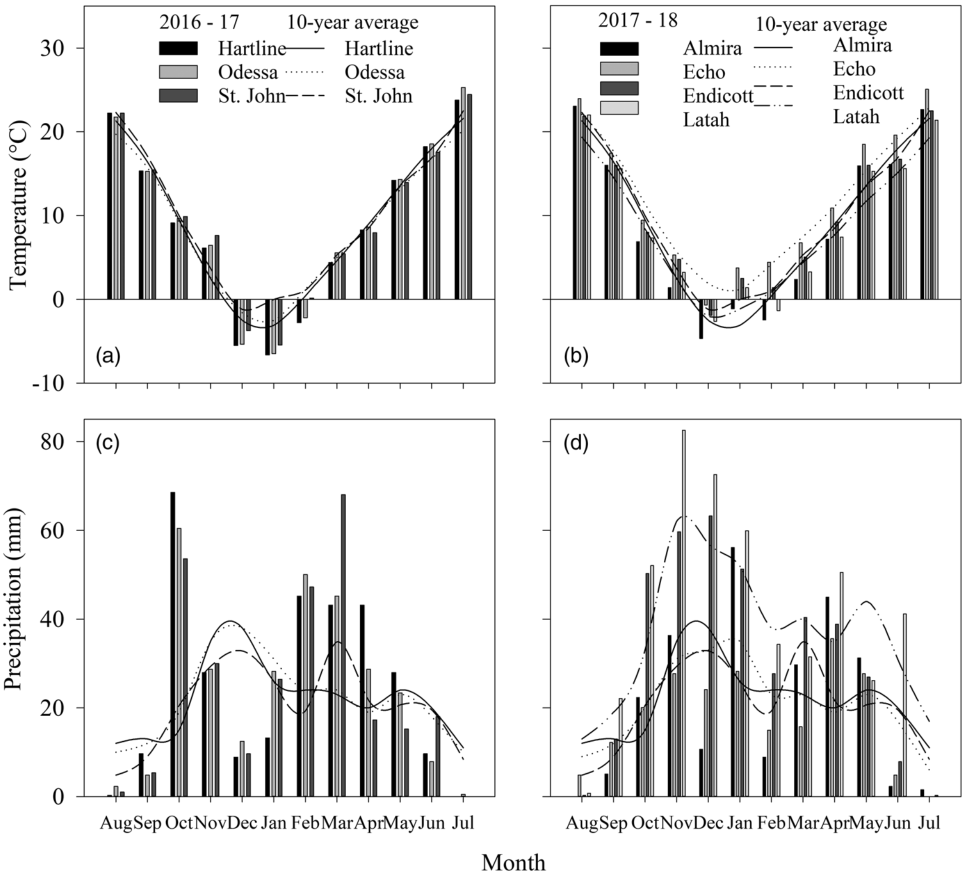

FIGURE 1 (a and b) Monthly average temperatures during the 2016–2017 (a) and 2017–2018 (b) crop years compared with their recent 10-yr monthly averages. (c and d) Monthly total precipitation during the 2016–2017 (c) and 2017–2018 (d) crop years compared with their recent 10-yr total monthly averages.

where *A* is the yield potential for individual site years, WUE is crop water use efficiency, and H_2O_0 is the minimum water threshold for biomass production (Pan et al., 2016a). For SC production in the iPNW, H_2O_0 of 61 mm was used, which was the minimum water threshold for wheat production in eastern Washington (Schillinger, Schofstoll, & Alldredge, 2008). Total N supply was determined as:

$$\text{total N supply} = (\text{preplant fall, root zone inorganic N}) + (\text{estimated mineralized N}) + (\text{fertilizer N})$$

The amount of mineralized N was estimated as percent organic matter in the top 30 cm of soil multiplied by 19 kg N ha^{-1} (Koenig, 2005; Pan et al., 2016a). Replicated yield responses to total N supply for individual site years were fitted first to a linear model and then to the Mitscherlich growth factor response model using Sigmaplot (Systat Software, Inc.). The Mitscherlich growth factor response model is defined as:

$$Y = A(1 - 10^{-cx})$$

where *Y* is yield (kg ha^{-1}), *x* is the N supply rate (kg N ha^{-1}), *A* is maximum yield (kg ha^{-1}), and c is the efficiency constant (Mitscherlich, 1909). Linear models were not significant for any site.

Analysis of variance of yield, protein, and oil concentrations was performed using Proc GLIMMIX in SAS statistical programming (SAS, 2008). The fixed effects were site-year and N fertilizer rate and timing and the interaction of fertilizer rate and timing. Block was treated as a random affect. Because site-year had significant effects on yield, oil concentration, and protein concentration (Table 3) and because of differences in treatments in the two crop years, data were analyzed independently for each year-site using the Proc Mixed procedure. Comparison of WC oil and protein concentrations among N rate treatments within each N application timing were analyzed using the Proc GLIMMIX procedure. The Covtest statement with homogeneity option was used to examine homogeneity of covariance parameters across sites and N timing. All figures were created in SigmaPlot 12.3 (Systat Software Inc., 2011). The linear relationships between total available water and maximum yield and between average maximum temperature and seed oil/protein concentration ratio

TABLE 3 Significance of effects of year, site, N fertilization rate, and timing on winter canola yield properties

	Source							
	Year-site		Timing		Rate		Timing × rate	
	df	*P*-value	df	*P*-value	df	*P*-value	df	*P*-value
Yield	6	<.0001	3	ns	16	ns	26	ns
Oil concentration	6	<.0001	3	0.0027	16	<.0001	26	0.0006
Protein concentration	6	<.0001	3	0.0030	16	<.0001	26	0.0288

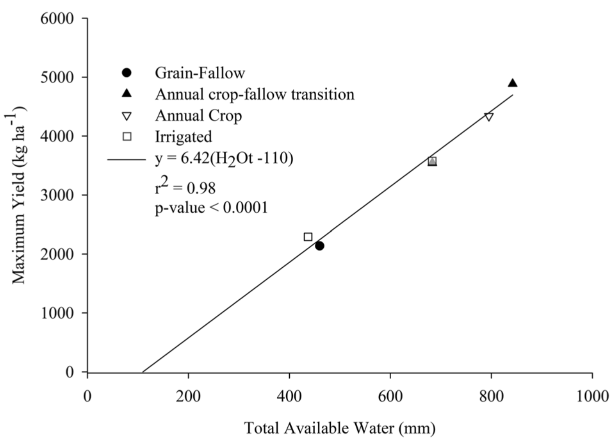

FIGURE 2 Relationship between total available water (fall stored soil water + precipitation + irrigation) and maximum yield of winter canola.

were plotted using site average data; all other figures were created using individual plot data.

3 | RESULTS AND DISCUSSION

3.1 | Weather conditions and total available water

The 2016–2017 winter was colder than normal, with average air temperatures in December and January dropping below 5°C (Figure 1a). Above-average precipitation occurred in October, February, and March at all three sites, whereas December was drier than normal (Figure 1c). The 2017–2018 winter was warmer and wetter than normal, with high precipitation occurring at Endicott and Latah in November through January (Figure 1b,d). Total available water ranged from 353 mm at Hartline in the grain–fallow AEC to 842 mm at Endicott in the grain-fallow transition AEC (Table 3).

3.2 | Soil nitrogen and soil water effects on yield

Soil test residual inorganic N and N mineralization estimates were summed to estimate soil N supply before fertilizer application. Soil N supply was high at all sites (range, 92–224 kg N ha^{-1}) (Table 4). High soil residual N is somewhat common in fields of the region. Yields of dryland WC were highly variable (range, 1778–4887 kg ha^{-1}). The highest yields were obtained in the annual crop AEC, followed by the annual crop–fallow transition AE and the grain–fallow AEC (Table 4). At Hartline in 2017, high seed loss occurred at harvest due to combine malfunction; therefore, yield data from that site are not included. Odessa yields were abnormally low for an irrigated site. In comparison, yields at Echo were more representative of the expected yields under irrigated conditions. Infestations of tumble mustard (*Sisymbrium altissimum* L.) and tansy mustard (*Descurainia pinnata*) were present in the Odessa field, which likely contributed to the reduced yields. No disease in any fields was observed or reported by farmers.

Results suggest that total available water was one of the primary drivers of WC maximum yield (Figure 2). In this WC study, we found the slope of the relationship equal to a 6.42-kg seed increase per each millimeter increase in available water (r^2 = .98). The x-intercept, which can be considered the theoretical minimum water required to produce a canola crop, was 110 mm, which is greater than the threshold for wheat production (Schillinger et al., 2008). The result showed a similar water–yield relationship as SC. Pan et al. (2016) showed SC seed yield to increase by 3.2 kg for each millimeter increase in available water. The higher slope for WC reflects a greater WUE of WC compared with SC. This greater WUE can be attributed to WC taking advantage of fall and winter precipitation, which is when 70% annual precipitation occurs in the iPNW.

Yields plotted as a function of total N supply typically follow the law of diminishing returns. That is, the yield response to the supply of a limited essential nutrient follows a diminishing exponential rise to a maximum yield, at which the nutrient is no longer limiting (Mitscherlich, 1909). The N-supply–based yield response curve intersects the origin under the assumption that yield is zero in the absence of an essential nutrient. Maximum yields for each site-year were obtained by forcing Mitscherlich response curves through zero. Only at one of seven sites was the Mitscherlich model statistically significant (Figure 3). At Odessa, where the yield was lowest and total N supply was below 100 kg ha^{-1}, yield response to total N supply plateaued at 143 kg N ha^{-1} (Figure 3). At the other

TABLE 4 Agroecological class (AEC), available soil water to 180 cm, precipitation from fall soil sampling through harvest, total available water (H_2Ot), inorganic soil N, estimated N mineralization, N supply, and seed yield at each winter canola site by year

Site-year	AEC[a]	Soil H_2O	Precipitation[b]	H_2Ot	Inorganic N[c]	Mineralized N[d]	Soil N supply[e]	Seed yield
		mm			kg N ha^{-1}			kg ha^{-1}
2016–2017								
Hartline	GF	37	297	334	90	15	105	ND[f]
Odessa	IR	37	400[b]	437	53	38	92	2291
St. John	AFT	264	417	681	152	54	207	3574
2017–2018								
Almira	GF	238	222	460	94	21	116	2137
Echo	IR	93	590[b]	683	86	17	103	3543
Endicott	AFT	476	366	842	113	27	141	4887
Latah	AC	344	471	795	182	42	224	4340

[a]AC, annual crop; AFT, annual crop–fallow transition; GF, grain–fallow; IR, irrigated.
[b]Precipitation + irrigation.
[c]$NO_3^+ + NH_4^+$ to 180 cm depth.
[d]Percentage of organic matter in top 30 cm × 19 kg N kg^{-1}.
[e]Soil N supply = Inorganic N + Mineralized N.
[f]No data due to combine malfunction at harvest.

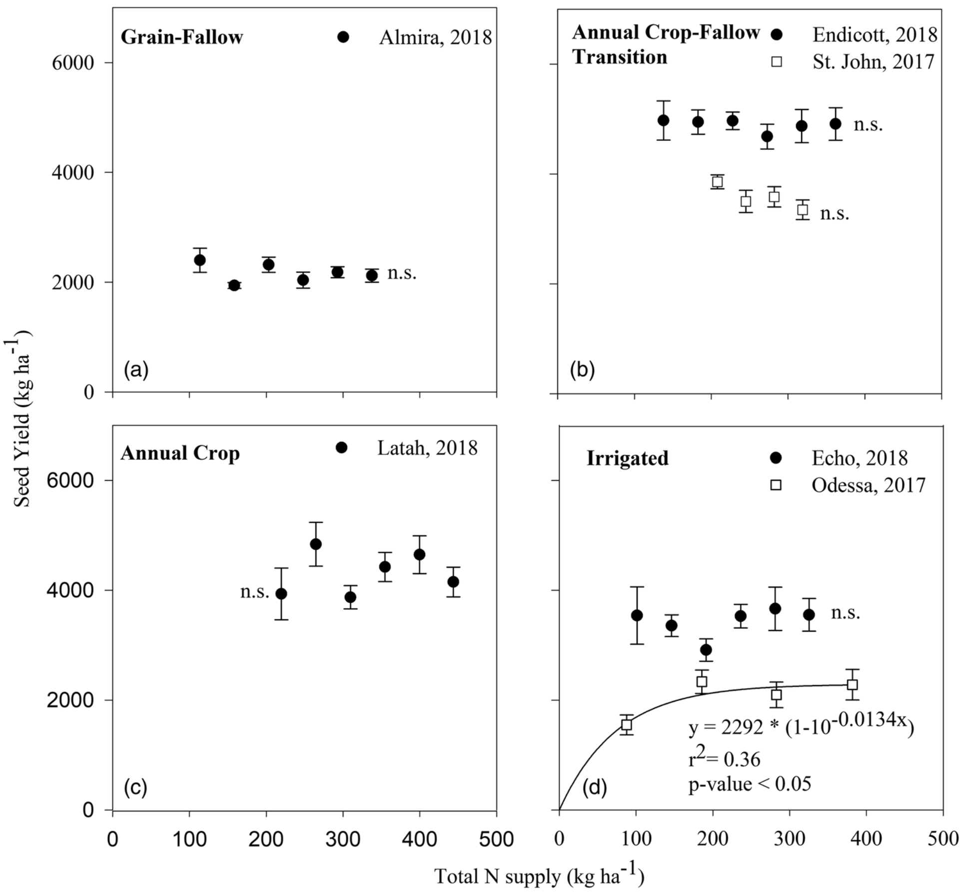

FIGURE 3 Winter canola yield response to total N supply and equations determining maximum winter canola yield at sites across the four agroecological classes: (a) grain–fallow, (b) annual crop–fallow transition, (c) annual crop, and (d) irrigated during the 2016–2017 and 2017–2018 crop years. Data points represent mean yields at replicated fertilizer rates. Yields associated with different timings of N fertilizer applications were averaged across the different rates. Error bars represent SEM yield at each total N supply. n.s., model was not significant at $p < .05$.

sites, N was not limiting because the soil N supply was sufficient to produce high yields without additional N fertilizer (Figure 3).

These findings are similar to the results of an N-rate study on SC in Washington, where a yield response to increasing N supply was observed at only one of five sites when N supply was above 100 kg N ha^{-1} (Pan et al., 2016a). At SC sites where N supply was below 100 kg ha^{-1}, a yield response to increasing N supply was observed at six of seven sites (Pan et al., 2016a). In winter rapeseed studies, yield response to N supply was observed to peak between 90 and 200 kg N ha^{-1} (Aminpanah, 2013; Cheema, Basra, Shah, Hussain, & Malik, 2001; Ferguson, Chastain, Garbacik, Chastain, & Wysocki, 2016; Ozturk, 2010).

High N uptake with high N rates can induce lodging, which reduces yield and/or causes harvest difficulties (Ferguson et al., 2016). This phenomenon was observed in our plots at St. John, Latah, and Endicott. Yield variation within each treatment was likely due to uneven stand establishment observed in fall and soil moisture availability differences in fall within a site. Lack of yield response can be attributed to soil N values at planting being greater than critical levels at which N is limiting (Table 2; Figure 3).

3.3 | Nitrogen application rate and timing effects on seed quality

Canola grew in different sites and years had significantly different oil and protein concentrations. Across all site-years, N application rate and timing had significant effects on seed oil and protein concentrations (Table 5). Fall application resulted in the greatest oil concentrations and the smallest protein concentrations, whereas spring or split applications resulted in similar oil and protein concentrations. In general, higher N application rates lead to lower oil and higher protein concentrations in the seeds. The effects of N application rate and timing on seed oil and protein concentrations were inconsistent among the seven year-sites. At five of seven year-sites, N application rate had a significant effect on seed oil and protein concentrations at the $\alpha = .05$ level. At two of seven year-sites, N application timing had a significant effect on seed oil and protein concentrations at the $\alpha = .1$ level (Table 6). Further analysis focused on the significant effects of N application rate at each timing for each year-site (Tables 7 and 8). At Almira and Latah, as N rate increased, protein concentration increased and oil concentration decreased across all N timings, except for spring-applied N at Latah. At Endicott, protein concentration increased with increasing N rate only in response to spring-applied N. Similarly, at St. John, oil concentration decreased with increasing N rate only in response to spring-applied N. When compared across sites, the increase in protein concentration was stronger than the decrease in oil

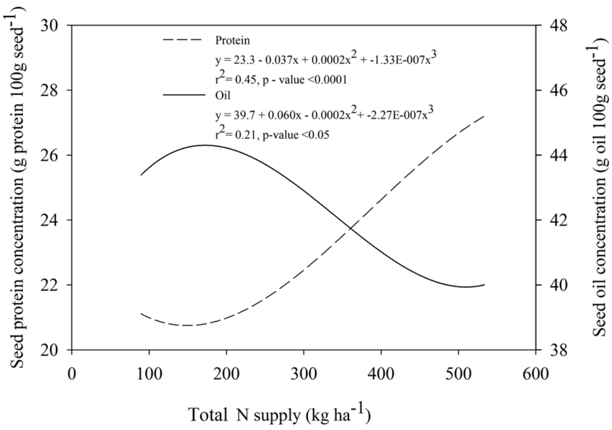

FIGURE 4 Seed protein and oil concentrations in response to N supply (fall preplant N + fertilizer N + estimated mineralized N) during the 2016–2017 and 2017–2018 crop years at seven winter canola field sites (n = 332).

concentration in response to increased N supply (Figure 4). The response of oil and protein concentrations to N fertilizer rates in the absence of N yield response has also been reported by Gao et al. (2010), Karamanos, Goh, and Flaten (2011), and Rathke, Christen, and Diepenbrock (2005). Variable oil and protein response to N applications can be attributed to different cultivars responding differently to N applications and to temperature and water stresses that can also influence oil and protein concentrations (Aminpanah, 2013; Hocking & Stapper, 2001; Mason & Brennan, 1998).

Seed protein concentration decreased as N application increased to ∼200 kg N ha^{-1} but increased dramatically as N supply increased from 200 to 500 kg N ha^{-1}. Seed oil concentration increased as N supply increased up to 200 kg N ha^{-1} but decreased dramatically as N supply increased from 200 to 500 kg N ha^{-1} (Figure 4). Our finding of an increase in protein and a corresponding decrease in oil concentration in response to increasing N supply is consistent with other reports (Asare & Scarisbrick, 1995; Cheema et al., 2001; Gao et al., 2010; Hocking, Randall, & DeMarco, 1997; Karamanos et al., 2011; Kirkegaard, Hocking, Angus, Howe, & Gardner, 1997; Mason & Brennan, 1998; Rathke et al., 2005). Ozturk (2010) also documented a quadratic relationship between N supply and oil concentration. Hocking et al. (1997) attributed the inverse relationship between N rate and oil concentration to a reduced availability of carbohydrates for oil synthesis at high N rates. Furthermore, Gao et al. (2010) found that urea additions changed the seed fatty acid profile, increasing the less desirable total saturated fatty acid concentration (palmatic + stearic + arachidic acid) and decreasing the oil quality index ratio, defined as (oleic acid)/(linoleic + linolenic acid).

This study is the first to document seed oil and protein concentration response to N application timing. We found that timing of N application had a significant effect on seed oil

TABLE 5 Seed oil and protein concentrations in response to different N rates and timings at the seven winter canola site-years during the 2016–2018 crop years[a]

Parameter	Category[b]	Oil mean g oil 100 g seed^{-1}	Protein mean g protein 100 g seed^{-1}
N timing**	fall	43.3a	22.1b
	split	42.2b	22.5ab
	spring	42.2b	22.8a
N rate, kg N ha^{-1}**	0	44.5a	21.1b
	16	43.1abc	22.6ab
	32	42.6abc	22.1ab
	37	43.8ab	21.9ab
	45	43.5ab	21.6b
	48	43.2abc	21.6ab
	74	42.6abc	22.7ab
	90	43.6ab	21.7ab
	98	42.4abc	22.5ab
	111	42.6abc	22.7ab
	135	42.3bc	23.1a
	180	41.9bc	23.3a
	195	41.2bc	23.0ab
	224	41.8bc	23.5a
	294	39.6c	24.2a
Site × year**			
St. John 2016		45.6a	19.4c
Latah 2017		44.6ab	22.6b
Almira 2017		43.2bc	22.6b
Hartline 2016		42.5bcd	22.3bc
Odessa 2016		41.9cde	20.7cd
Endicott 2017		40.2de	26.1a
Echo 2017		40.0e	23.5b

[a]Means followed by the same letter are not significantly different at $p < .10$ at the Almira year-site and at $p < .05$ for all other year-sites within each N application timing.
[b]Fall: 100% fertilizer N was applied in fall after seeding; split: 50% fertilizer N was applied in fall and 50% was applied in spring; spring: 100% fertilizer N was applied in March to April. Rates varied across sites in 2016–2017 crop year based on the yield goal method; rates were 0, 45, 90, 135, 180, 224 kg N ha^{-1}.
**Significant at .01 probability level.

TABLE 6 Significance of effects of N fertilization rate and timing on winter canola seed oil and protein concentrations

		df		*P*-value			
				Oil concentration		Protein concentration	
Year	Site	Rate	Timing	Rate	Timing	Rate	Timing
2016–2017	Hartline	3	2	ns[a]	ns	ns	ns
	Odessa	3	2	.0026	ns	.0058	ns
	St. John	3	2	.0115	ns	.0080	ns
2017–2018	Almira	5	2	<.0001	ns	<.0001	ns
	Echo	5	2	ns	.0674	ns	.0584
	Endicott	5	2	.0005	ns	.0004	ns
	Latah	5	2	<.0001	ns	<.0001	.0644

[a]Not significant.

TABLE 7 Seed oil and protein concentrations in response to different N rates and timings at the seven winter canola field sites during the 2016–2017 crop year[a]

Timing[b]	N rate[c] kg N ha^{-1}	Hartline	Odessa	St. John	Hartline	Odessa	St. John
		g protein 100 g seed^{-1}			g oil 100 g seed^{-1}		
Fall	0	22.1	20.0	18.6	44.0	42.9	46.9
	16/98/37	20.5	21.2	18.6	44.3	40.7	46.7
	32/195/74	21.6	21.1	19.0	44.2	40.7	46.4
	48/294/111	19.4	22.3	18.6	45.4	39.2	47.1
Split	0	21.0	19.3	17.6b	44.7	43.6	48.0
	16/98/37	24.3	20.8	19.3ab	41.4	41.3	46.4
	32/195/74	24.2	21.7	19.2ab	37.9	39.7	46.5
	48/294/111	22.1	21.5	20.0a	44.2	40.0	45.5
Spring	0	19.8	18.8b	18.0b	44.5	44.8a	47.5a
	16/98/37	22.4	20.2ab	18.5ab	43.4	43.1ab	47.2a
	32/195/74	19.9	20.9ab	20.6a	45.3	41.0ab	43.9b
	48/294/111	22.8	23.5a	20.3a	39.7	37.5b	44.2b

[a]Means followed by the same letter are not significantly different at $p < .1$ at Almira year-site and at $p < .05$ for all other year-sites within each N application timing.
[b]Fall: 100% fertilizer N was applied in fall after seeding; split: 50% fertilizer N was applied. in fall and 50% was applied in spring; spring: 100% fertilizer N was applied in March to April.
[c]Rates varied across sites based on the yield goal method; displayed as Hartline/Odessa/St. John.

TABLE 8 Seed oil and protein concentrations in response to different N rates and timings at the seven winter canola field sites during the 2017–2018 crop year[a]

Timing[b]	N rate[c] kg N ha^{-1}	Almira	Echo	Endicott	Latah	Almira	Echo	Endicott	Latah
		g protein 100 g seed^{-1}				g oil 100 g seed^{-1}			
Fall	0	21.3b	23.1	25.0	19.9c	45.3a	41.0	42.0	47.5a
	45	21.6b	21.6	25.2	21.0bc	44.3ab	42.0	41.0	46.5ab
	90	21.9b	21.4	26.0	20.2c	45.3a	43.7	41.2	46.2ab
	135	23.0ab	22.3	27.5	21.8abc	43.3ab	42.6	40.3	46.1ab
	180	22.5ab	21.5	26.4	24.2a	43.8ab	43.1	39.8	43.0c
	224	24.2a	21.7	26.8	23.3ab	41.7b	42.7	40.2	44.2bc
Split	0	21.3b	23.1	25.0	20.0c	45.3a	41.0	42.0	47.6a
	45	21.6b	22.1	24.8	23.0 abc	44.5ab	41.9	41.2	44.8abc
	90	21.4b	23.7	24.7	20.2bc	44.4ab	39.9	42.4	47.2ab
	135	23.6a	23.7	26.8	23.8ab	42.9ab	40.0	40.4	43.1c
	180	23.8a	24.1	26.3	24.3a	42.1b	39.5	40.3	43.1c
	224	23.8a	24.9	26.7	23.8ab	42.0b	38.0	40.0	43.5bc
Spring	0	21.3c	23.1	25.0bc	20.0b	45.3a	41.0	42.0a	47.5a
	45	21.2c	24.1	24.5c	23.6ab	44.9a	38.9	41.5ab	43.8ab
	90	21.5bc	24.1	26.4ab	23.8a	44.2ab	39.5	40.6ab	43.5b
	135	23.2ab	25.2	25.7bc	24.6a	42.2bc	38.3	40.2ab	43.4b
	180	23.8a	25.5	27.5a	24.6a	41.2c	37.8	39.0b	44.0ab
	224	24.2a	24.9	27.8a	24.1a	41.9bc	38.0	39.6b	43.7b

[a]Means followed by the same letter are not significantly different at $p < .1$ at Almira year-site and at $p < .05$ for all other year-sites within each N application timing.
[b]Fall: 100% fertilizer N was applied in fall after seeding; split: 50% fertilizer N was applied. in fall and 50% was applied in spring; spring: 100% fertilizer N was applied in March to April.
[c]Rates varied across sites based on the yield goal method; displayed as Hartline/Odessa/St. John.

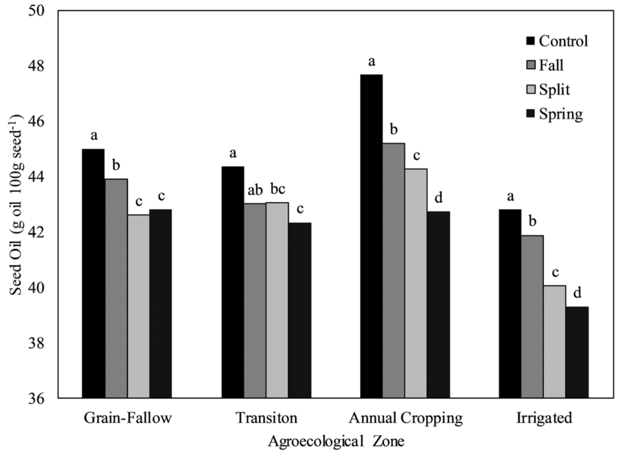

FIGURE 5 The relationship between mean seed oil concentration and timing of N application for the different agroecological classes. Data for each time of application were averaged across N rates. Seed oil concentration marked by different letters above the bars are significantly different within each agroecological class (n = 16–28 for each timing of N application in each agroecological zone).

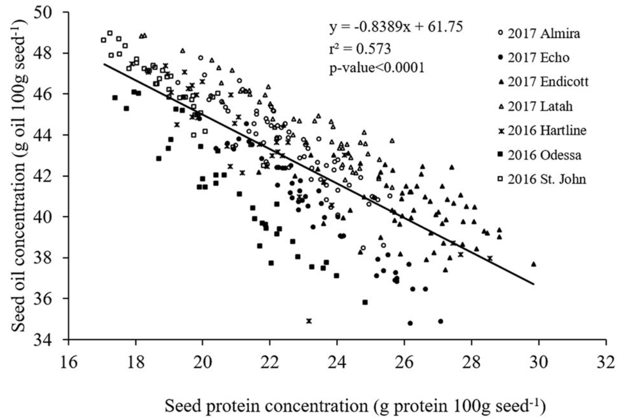

FIGURE 6 The inverse relationship between winter canola seed oil and protein concentration. Data points represent all treatment combinations from seven sites in the 2016–2017 and 2017–2018 crop years.

concentration, with different responses observed across the AECs (Figure 5). Oil concentration was significantly higher in control than all other treatments across all AECs. In the grain–fallow, annual, and irrigated cropping zones, fall N application resulted in the highest oil concentrations compared with both split and spring N applications. In the transition zone, fall-applied N resulted in higher oil concentrations than spring-applied N. We found the lowest seed oil concentrations with spring-applied N in all AECs. The annual cropping zone experienced the greatest reduction in response to the spring application, with oil concentration decreasing by 10% compared with the control. In the annual and irrigated zones, we also found significant differences in oil concentration response between split and spring N applications. This finding may be due to higher soil water availability and corresponding N leaching losses at the time of fall-applied N in the split treatment. Consequently, sites with spring-applied N only had a greater amount of available N for crop uptake and, thereby, a stronger oil concentration response.

3.4 | Relationship between canola seed oil and protein

Winter canola oil and protein concentrations exhibited an inverse linear relationship across all site years, described by the following equation: oil concentration = 61.75 − 0.8389 × crude protein concentration (r^2 = .57; n = 332) (Figure 6). This relationship is similar to that described by Mason and Brennan (1998), who first reported the linear relationship: oil concentration = 57.68 − 0.8474 × crude protein concentration (r^2 = .74), and by Rathke et al. (2005), who found the relationship at a site in Germany: oil concentration = 64.67 − 0.9667 × crude protein concentration (r^2 = .81; n = 192). The higher r^2 value in Rathke et al. (2005) could be attributed to the data being from the same site location and cultivar across years. In our study, when we observed the relationship independently for each site, the r^2 values ranged from 0.33 to 0.95. Mitra and Bhatia (1979) explained the physiological reason for the reversed relationship between oil and protein concentration as the competition for carbohydrate skeletons during protein and fatty acid metabolism. Protein and fatty acid synthesis both require carbon compounds produced from the decomposition of carbohydrates. Increased N supply enhances synthesis of proteins at the expense of fatty acid synthesis, resulting in a lower oil concentration (Mitra & Bhatia, 1979).

3.5 | Influences of air temperature on seed quality

Climatic conditions influence canola oil and protein production (Hammac et al., 2017; Hocking & Stapper, 2001; Kirkegaard et al., 1997; Ozturk, 2010). For example, decreases in oil concentration by 1–2.7% per 1°C increase in mean temperature during the seed filling stage have been reported (Hocking & Stapper, 2001; Kirkegaard et al., 1997). In the iPNW, Hammac et al. (2017) found that total oil and protein concentration was explained by total available water and temperature stress during flowering. In this context, temperature stress is defined as the number of days during flowering when maximum air temperature reached 28°C or greater; these are the temperatures at which Aksouh-Harradj, Campbell, and Mailer (2006) found canola yield to decrease and seed fatty-acid profile to change. We found no relationship

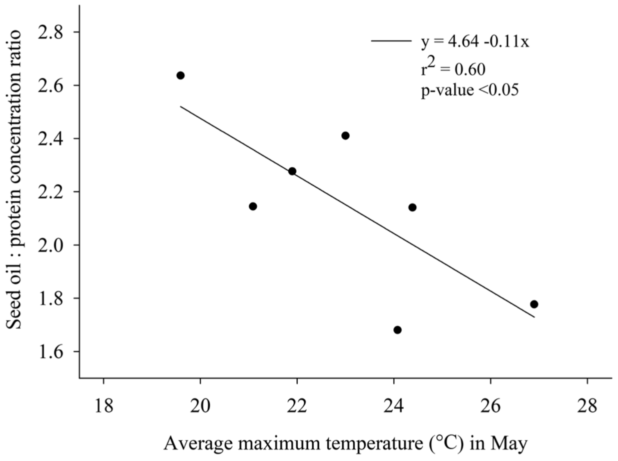

FIGURE 7 Average seed oil/protein ratio in response to average maximum temperature in May at winter canola sites in the 2016–2017 and 2017–2018 crop years.

between oil, protein, or their ratio with total available water as reported in Hammac et al. (2017). However, the average seed oil/protein ratio at each site decreased as average maximum air temperature increased during May, when flowering occurred (Figure 7). Exact start and end dates of flowering were not recorded, but flowering in May was observed at all sites. Furthermore, the relationship between May's average maximum temperature and seed oil/protein ratio ($r^2 = .60$) was stronger than between temperature and oil concentration ($r^2 = .45$) or temperature and protein concentration ($r^2 = .54$), analyzed individually.

Because WC flowers much earlier than SC, it is less susceptible to oil-reducing heat stress and is a better choice for farmers in regions that consistently experience high temperatures in late spring. With climate change models for the iPNW predicting increased winter precipitation and warmer and drier spring months, WC is better adapted to take advantage of winter precipitation and to avoid the negative influences of the heat in late spring (Stöckle et al., 2018).

4 | CONCLUSIONS

We found that WC yield in the iPNW was influenced mainly by total available water, which is similar to SC yield response in the region. Winter canola yield was highest in the annual crop and annual crop–fallow transition AECs. Nitrogen fertilizer was a factor in yield response only when soil N supply was <100 kg N ha^{-1}. When N supply was >100 kg N ha^{-1}, WC produced high yields without additional N. Higher N rates resulted in lower oil concentrations and higher protein concentrations, with these relationships being strongest for spring-applied N in the grain–fallow transition AEC, followed by annual cropping and irrigated AECs. These findings emphasize the need to consider soil N levels in fertilizer decisions. The current yield goal–based N recommendation should be modified to integrate the soil test threshold.

Canola produced in the iPNW is processed for both edible oil for human consumption and protein meal for livestock and fish feed. Oil and protein premiums vary between years and should be considered in N management decisions, based on our finding of opposite responses of oil and protein concentrations to N rate and timing. Moderating N rates and minimizing late-season applications may help meet oil premiums, whereas applying N later in the season will increase protein concentrations.

Additionally, heat stress reduces oil concentration and increases protein concentration, as seen in a reduction in the seed oil/protein ratio with increasing temperatures during flowering. Consequently, premiums and end-use quality should also be considered when deciding whether to plant WC or SC. Because it flowers earlier than SC, WC may be a better choice in regions that experience high temperatures in late spring.

ACKNOWLEDGMENTS

This work was supported by Washington Oilseeds Cropping Systems Project and USDA National Institute of Food and Agriculture (Hatch project 1014527). We thank the participating farmers for their generosity in providing land and site management and Dr. Linda R. Klein for valuable writing advice and editing the manuscript.

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How to cite this article: Porter MJ, Pan WL, Schillinger WF, Madsen IJ, Sowers KE, Tao H. Winter Canola Response to Soil and Fertilizer Nitrogen in Semiarid Mediterranean Conditions. *Agronomy Journal*. 2020;1–14. https://doi.org/10.1002/agj2.20119