1. Project title and ADF file number.

ADF File No. 20170288: *Revisiting nitrogen fertilizer recommendations for Saskatchewan: Are we measuring the right soil nitrogen pool?*

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4. Abstract/Summary

Autoclaved citrate extractable (ACE) soil protein is believed to be related to the ability of a soil to make nitrogen (N) available for plant uptake through mineralization. However, the ACE extraction method is neither rapid nor compatible with the high-throughput demands of commercial soil testing labs. Thus, the objectives of this study were to (i) develop a rapid, microwave-assisted citrate extraction method to determine soil protein; and (ii) evaluate the citrate extractable soil protein pool as a predictor of grain yield and N uptake by wheat and canola in a wide range of field soils. Soils (n = 55) from across the province were collected in fall 2018 and soil protein determinations were made using the standard ACE method. ACE soil protein was highly correlated with total soil N (r = 0.820***) but was not correlated with potentially mineralizable N (PMN: determined using a 7-day anaerobic incubation). Whereas soil protein N accounted for a significant fraction (*ca*. 50%) of the total N in these soils, PMN accounted for only about 1% of total soil N. Together, these data indicate that—at least over the short-term—only a small fraction (1.5–2.3%) of the N extracted as ACE soil protein is biologically available. A rapid (15-min) microwave-assisted citrate extraction (MACE) method was developed that yielded soil protein concentrations comparable (R^2 = 0.967***) to those obtained using the standard *ACE* method. Overall, however, our results failed to demonstrate that the soil protein pool extracted using the ACE/MACE methodology could a useful measure of the N that potentially becomes biologically available during the season.

Fertilizer response studies were conducted in 2019 and 2020 at the AgriARM sites in Prince Albert (CLC), Melfort (NARF), Outlook (ICDC), Indian Head (IHARF), Swift Current (WCA), Scott (WARC), and Yorkton (ECRF). Although the response of wheat and canola to fertilizer N varied between years and sites, trends suggested that the largest response in wheat and canola yield occurred with the first increment of applied N (i.e., the 0.5× the soil test recommendation) with further increases occurring at the 1× soil test recommendation rate. Statistically significant yield increases often were not observed above the 1× rate, suggesting that current soil test recommendations provide an acceptable measure of biologically available N, with the understanding that the vagaries of weather strongly influence N responses.

5. Extension Messages

- *Microwave-assisted citrate extraction* (MACE) provides a viable and more rapid alternative to *autoclaved citrate extraction* (ACE) for soil protein determinations.
- Citrate extractable soil protein either was not correlated with yield of wheat and canola or was only weakly negatively correlated with crop yield. This outcome suggests that the soil protein pool extracted using the ACE/MACE methodology is not an appropriate measure of the N that potentially becomes biologically available during the season. We found that these methods extract about 40–50% of the total organic N in soils while only about 1–2% of total soil N was potentially mineralizable. Thus, it appears that although the pool of soil organic N extracted using the ACE/MACE methodology may include potentially mineralizable N, any useful measure of potentially biologically available N is obscured by the large size of the ACE/MACE soil protein pool. Consequently, we conclude that ACE/MACE measures of soil protein are not suitable as a short-term (seasonal) N availability index.

6. Introduction

Nitrogen (N) is one of the most biologically and economically important plant nutrients and, along with water, is the factor that most frequently limits crop production. In recent years, however, soil N tests have come under considerable scrutiny and criticism, leading some to suggest that the lack of an appropriate soil N test is one of the biggest limitations to developing appropriate fertilizer N recommendations and, hence sustainable N use. Indeed, Les Henry (Grainews; September 17, 2015) has stated that "the biggest current limitation to soil testing is a lack of a test for N that will be mineralized during the growing season." When we test a soil for N, we are really asking the question, how much N is this soil likely to supply during the growing season? Curiously, however, what we typically measure is the product of mineralization—specifically soil inorganic N—and then build in some fudge factors to predict how much additional N is likely to be released, given the soil zone (Black soils, which contain more organic matter and more likely to release N than a Brown soil, etc.).

If we want to know how much N can be mineralized, why not simply let the soil mineralize and measure the outcome? The answer lies in the many impracticalities that make this approach untenable. For example, under what temperature conditions should the soil be held? For how long? Does it matter if the soil is more, or less, moist? (it does!). What happens if the soil goes anaerobic, or worse, if it dries out? Ultimately, measuring the product of mineralization is problematic, unless the incubation conditions are tightly controlled and reproducible. As an alternative, many studies have examined various N availability indices over the years, but despite these many studies, no single test has been identified that has been widely adopted (e.g., Martinez et al. 2017; Schomberg et al. 2009; Walley et al. 2002). The difficulty reflects, in part, the need to develop an affordable, easily reproducible, and chemically defensible test that measures the substrate for N mineralization (i.e., soil protein), and not the product (inorganic N). This approach is further supported by the emerging understanding that organic N sources, such as amino acids released on depolymerization of soil proteins, are available for plant N uptake (Schimel and Bennett 2004), and the degree to which these sources of N contribute to the available N pool that the plant "sees" depends on the size of the inorganic soil N. Moreover, Schimel and Bennett (2004) argue that N-cycling is driven by the depolymerization of N-containing polymers (i.e., soil proteins) and thus soil proteins are the key substrate that ultimately contributes to the plant available N pool which contains both organic monomers (not measured by typical soil N tests) and inorganic N. Indeed, soil N tests currently in use in western Canada (including Saskatchewan) generally involve the use of a chemical extractant to determine the amount of "plant available" (i.e., nitrate) N in the soil or short-term incubation release (e.g., PRS probes). Ultimately, however, these methods provide only a "snapshot" of the

amount of inorganic N (nitrate and/or ammonium) either in the inorganic N pool or entering this pool. Potentially mineralizable N, on the other hand, represents the N in the soil organic N pool that may become available during the growing season (Campbell et al., 1995) and proteins represent the largest pool of organic nitrogen (N) in soils (Nannipieri and Paul, 2009). Thus, soil proteins also represent a reservoir of potentially mineralizable N (Moebius-Clune et al., 2016).

In addition to being considered a critical indicator of soil biological health (Hurisso et al., 2018; Wu and Congreves, 2021), there is increasing evidence that soil protein—and importantly, the depolymerization of this soil protein—provides a measure of plant available N (Enggrob et al., 2019; Martin & Sprunger, 2021). Moreover, our research has identified a soil protein pool (easily-extractable glomalin-related soil protein) that we believe is representative of potentially mineralizable N that can be rapidly measured in the laboratory. The standard method for extracting soil protein is based on methods developed by Wright and co-workers (Wright et al., 2006; Wright and Upadhyaya, 1998, 1996) for the extraction of glomalin—a glycoprotein produced by arbuscular mycorrhizal fungi—from soils and is often referred to as easily extractable glomalin-related soil protein (*EE-GRSP*) or, more recently, Autoclaved Citrate Extractable (*ACE*) protein (Hurisso et al., 2018). Essentially, the method involves autoclaving soil in a 20 mM sodium citrate buffer (pH 7.0) for 30 min followed by a bicinchoninic acid (BCA) assay (Smith et al., 1985) for protein quantification. As a result of the requirement for autoclaving the soil/citrate slurry, the *ACE* method is neither rapid nor compatible with the high-throughput environment of commercial soil testing labs. For these reasons, *ACE* protein analysis currently is not regularly available in commercial soil tests.

On the other hand, most soil testing laboratories are equipped with microwave systems to facilitate sample digestion/extraction for elemental analysis. Microwave-assisted extraction methods also have been developed to extract bioactive and compounds and proteins from plant materials (Destandau et al., 2013; Flórez et al., 2015) and can provide the high temperature and pressure needed for soil protein extraction. Advantages of microwave-assisted extraction also include rapid heating and active cooling that could substantially reduce extraction times. Developing a rapid and less cumbersome method for extracting soil protein would make the test more widely accessible. Thus, the objectives of our research were to (i) determine whether microwave-assisted extraction of soil protein yields the same results as the standard *ACE* method; (ii) optimize the method; and (iii) demonstrate that this soil protein N pool is directly related to potentially mineralizable N and can provide a basis for improved fertilizer recommendations.

7. Objectives and progress towards meeting each objective

Although the project encountered an unavoidable delays due to the coronavirus pandemic, all phases of the project (i.e., field and laboratory experiments) have now been completed.

Objectives (list original/revised objectives)	**Status**
Develop a rapid soil test to determine mineralizable soil protein N (mSP-N)	Completed
Correlate mSP-N to potentially mineralizable soil N and relate to fertilizer recommendations	Completed

8. Methodology

Assessment of soil protein concentration in Saskatchewan soils: Soils (n = 55) from across the province were collected in fall 2018 and the soil physical and chemical characteristics of the soils determined using standard analytical methods (Carter & Gregorich, 2008). In addition, easily-extractable GRSP—hereafter referred to as *autoclaved citrate extractable* (*ACE*) protein—was determined using the method developed by Wright & Upadhyaya (1998) and modified by Hurisso et al. (2018). Briefly, 1.0 g samples of air-dried soil were weighed into 50-mL Falcon tubes to which 8 mL of 20 mM sodium citrate (pH = 7) was added. The tubes capped and vortexed for 20 s, the caps were then loosened, and the samples autoclaved at 121°C and 117 kPa for 30 min. The autoclaved samples were cooled to room temperature, centrifuged at 6,000 × g for 15 min, and sub-samples of the supernatant transferred into microtiter tubes and stored at 4°C overnight.

Microwave-assisted citrate extractable (MACE) soil protein was extracted by weighing 1.0 g of air-dried soil into 50-mL PTFE-TFM digestion/extraction vessels to which 8 mL of 20 mM sodium citrate (pH = 7) was added. The tubes were then capped and hand-shaken for 20 s. The reaction vessels were then assembled and placed in the cavity of an Ethos EX microwave extraction system (Milestone SRL, Italy) and the power adjusted to achieve and maintain a temperature of 121°C at 221 ± 10 kPa for 30 min (i.e., 500 W during the heating phase and 200–300 W during the temperature maintenance phase). The samples were then cooled to room temperature, quantitatively transferred into 50-mL centrifuge tubes, and centrifuged at 6,000 × g for 15 min. Sub-samples of the supernatant were then transferred into microtiter tubes and stored at 4°C overnight. Microwave-assisted extractions of each soil were repeated with the samples microwaved for 25, 20, 15, 10, or 5 min.

The soils (0–15 cm depth) used in this study were a subset (n = 7) of the samples used for soil health scoring research (Wu and Congreves, 2021) and were chosen to provide a range of texture, pH, soil organic C, and total N (Table 8.1). All samples were collected in the fall of 2018 from agricultural fields in Saskatchewan, Canada. Samples were collected using a 2.5 cm (i.d.) auger and were aired-dried and sieved through a 2-mm mesh screen prior to analysis.

Table 8.1. Key soil characteristics for the seven different soils used to evaluate methods of soil protein extraction.

Soil	Texture	pH	Soil organic C (g kg^{-1})	Total N (g kg^{-1})
1	loam	7.4	18.77	2.07
2	loam	7.6	21.44	2.75
3	loam	8.0	23.22	2.19
4	clay loam	5.8	31.16	2.93
5	clay loam	7.5	71.28	6.10
6	clay loam	8.2	22.59	2.43
7	loamy sand	8.4	5.35	0.39

Microwave-assisted citrate extraction of the soil protein was compared to the standard *ACE* method using duplicate samples extracted on each of three days during a one-week period (n = 84). For both the *ACE* and *MACE* extracts soil protein concentrations were determined within 24-h of extraction using the bicinchoninic acid (BCA) assay according to Schindelbeck et al. (2016) and Hurisso et al. (2018). The model protein, bovine serum albumin (BSA)—purchased as ready-to-use solutions (Thermo Fisher cat:23208)— was used as the standard. Calibration curves (absorbance *vs*. BSA concentration) were prepared following standard protocols (Hurisso et al., 2018) and were performed using a Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific). In general, the BCA method involved transferring 25 µl of extract or BSA solution into a microplate well, adding 200 µl of the BCA working solution, sealing the microplate, and incubating the samples at 37°C for 30 min. The absorbance at 562 nm was then measured using a microplate spectrophotometer (Bio Tek, Epoch™ 2). Soil protein concentrations were then converted to protein-N concentrations assuming the average N content of proteins is 16% (Jones, 1941; Geisseler et al., 2019):

$$ACE\text{-}PN = 0.16 \times ACE\text{-}P \quad (1)$$

where *ACE-PN* = *ACE* protein N (mg N g^{-1}), 0.16 = average N content of protein (mg N mg^{-1}); *ACE-P* = *ACE* protein (mg g^{-1}).

Fertilizer-N response studies: Field studies were conducted in 2019 and 2020 at the AgriARM sites in Prince Albert (Conservation Learning Centre; CLC), Melfort (Northeast Agriculture Research Foundation; NARF), Outlook (Irrigation Crop Diversification Corporation; ICDC), Indian Head (Indian Head Agricultural Research Foundation; IHARF), Swift Current

(Wheatland Conservation Area, Inc.; WCA), Scott (Western Applied Research Corporation; WARC), and Yorkton (East Central Research Foundation; ECRF). All field operations (e.g., seeding, fertilizer applications, plot maintenance, and harvest) were conducted by the local AgriARM personnel using the equipment available on-site, and followed a standard protocol developed in collaboration with the site managers. Crop management operations for the wheat and canola were site-specific (see Appendix, Tables A1 through A4) and followed best management practices for the region in which each site was located. The experimental design at each site involved establishing two sets of plots (one set for each crop) using a randomized complete block design with five treatments replicated four times. The treatments consisted of a non-fertilized control (0×) and fertilizer N applications equal to 0.5-, 1.0-, 1.5-, and 2.0-times (0.5×, 1×, 1.5×, and 2×) the soil test recommendation (STR = 1×)—with the actual amounts of fertilizer N applied being site- and crop-specific.

In preparation for the 2019 field season, soil samples (0–15, 15–30, and 30–60 cm depths) from each of the AgriARM sites were collected in fall 2018 and sent to a commercial soil testing laboratory (*FarmersEdge*; Winnipeg, MB) to obtain fertilizer recommendations. Results of the fall 2018 soil tests and the site- and crop-specific STRs for fertilizer N for the 2019 field season are summarized in Table 8.2. Research plots were established at each of the AgriARM sites in spring 2019, with canola grown at each site and wheat grown at six sites[1] (CLC, IHARF, NARF, ICDC, WARC, and WCA). At harvest, total above-ground biomass and seed yields were determined by harvesting the center five rows of the plots using a small-plot combine. Samples of the above-ground biomass and seed were collected and returned to the *Prairie Environmental Agronomy Research Laboratory* (*PEARL*) in the Department of Soil Science Department of Soil Science at the University of Saskatchewan for analysis (total N and C; oilseed content of canola).

Table 8.2. Soil test results and crop-specific soil test nitrogen (N) recommendations for the AgriARM sites.

Site (location)	Wheat		Canola		Wheat		Canola	
	2019 growing season				2020 growing season[a]			
	Soil mineral-N[b]	1× rate[c]	Soil mineral-N[b]	1× rate[c]	Soil mineral-N[b]	1× rate[c]	Soil mineral-N[b]	1× rate[c]
			kg N ha^{-1}					
East Central Research Foundation (ECRF; Yorkton)	- - -	- - -	22.4	120	29.1	130	- - -	- - -
Conservation Learning Centre (CLC; Prince Albert)	14.6	112	21.3	168	44.8	136	29.1	143
Indian Head Agricultural Research Foundation (IHARF; Indian Head)	21.3	130	29.1	130	14.6	110	10.1	110
Northeast Agriculture Research Foundation (NARF; Melfort)	19.0	124	41.4	124	47.1	120	50.4	147
Irrigation Crop Diversification Corporation (ICDC; Outlook)	19.4	130	14.6	130	20.2	136	- - -	- - -
Western Applied Research Corporation (WARC; Scott)	35.8	92	42.6	92	12.3	71	13.5	84
Wheatland Conservation Area, Inc. (WCA; Swift Current)	54.9	62	54.9	84	24.7	77	24.7	105

[a] For logistical reasons, soil test samples from ECRF, IHARF, WARC, and WCA were collected in fall 2019, whereas samples from ICDC and NARF were collected in spring 2020.

[b] Available soil N (i.e., NO_3^--N + NH_4^+-N) prior to seeding.

[c] The 1× rate is based on the amount of available N in the soil (0–30 cm) and the target yield for each crop/site combination.

In spring 2020, research plots at the AgriARM sites were moved to areas adjacent to the original (2019) plots, with wheat grown at all seven sites and canola grown at five sites[2] (CLC, IHARF, NARF, WARC, and WCA). Prior to seeding (i.e., in fall

[1] A communications error resulted in only a single crop (canola) being grown at the East Central Research Foundation (ECRF) site in 2019.

[2] Restrictions imposed in response to the coronavirus pandemic affected field operations in 2020 differently at each of the AgriARM sites, with the most significant impact being that only a single crop (wheat) was grown at the sites in Yorkton (ECRF) and Outlook (ICDC).

2019 or early spring 2020), soil samples (0–15, 15–30, and 30–60 cm depths) were collected from the areas in which the wheat and canola plots were to be established and sent to a commercial soil testing laboratory (*FarmersEdge*; Winnipeg, MB) to obtain fertilizer recommendations. Results of the fall 2019 and spring 2020 soil tests and the site- and crop-specific STRs for fertilizer N for the 2020 field season are summarized in Table 8.2. Mid-season biomass samples were collected by hand-harvesting two 1-m strips from adjacent rows outside the main harvest area (i.e., the centre 4–7 rows, depending on the size of the plots). At harvest, total above-ground biomass and seed yields were determined by harvesting the plots using a small-plot combine. Samples of the above-ground biomass and seed were collected and returned to the *PEARL* for processing and analysis (total N and C; oilseed content of canola; harvest index). Following the fall harvest, soil samples (0–15, 15–30, and 30–60 cm depths) were collected from the 0×, 1×, and 2× treatment plots (n = 102), returned to the *PEARL*, and processed and analyzed for various indices of soil N availability—including *ACE* soil protein and potentially mineralizable N—using standard analytical methods (Carter & Gregorich, 2008; Wright & Upadhyaya, 1996).

9. Results and discussion:

9.1 Development of a microwave-assisted extraction method for soil protein

Average soil protein concentrations obtained using the 30-min microwave-assisted extraction were comparable to those obtained using the standard ACE method—aligning close to the 1:1 line with an R^2 of 0.957 ($P < 0.001$) (Figure 9.1). Moreover, soil protein concentration was not affected by extraction method ($P = 0.369$). The *ACE* protein method is known to co-extract humic substances (Gillespie et al., 2011; Schindler et al., 2007) that can interfere with the BCA assay causing an overestimation of protein concentrations (Roberts & Jones, 2008) and in all probability these interfering substances also are extracted using the *MACE* method. This may account for the difference observed when soil protein concentrations were averaged across soils; i.e., MACE protein was about 6% greater than *ACE* protein (i.e., 5.74 mg g^{-1} *vs*. 5.42 mg g^{-1}). Because the Ethos EX system uses closed extraction vessels the extraction occurs at a higher pressure than that achieved during the autoclave method (i.e., 221 *vs*. 117 kPa), which may lead to more of the mineral-associated and membrane-bound protein—and more humic substances—being co-extracted using the *MACE* method. It has been reported that microwave extraction of proteins from rice bran did not affect the protein characteristics (Bedin et al., 2019); nevertheless, additional research will be needed to determine if or how soil proteins and the compounds co-extracted with soil protein are impacted by microwave-assisted extraction. Whereas our results demonstrated that a 30-min microwave-assisted citrate extraction can replace the standard autoclaved citrate extraction to determine soil protein, the next question was: can *MACE* soil protein extraction times be shortened without compromising the results?

The effect of extraction time on *MACE* soil protein concentration is shown in Figure 9.2. Soil protein concentrations were affected by extraction time ($P < 0.001$)—decreasing as the extraction time was decreased. However, at extraction times between 15 and 25 min the average *MACE* soil protein concentration was within 2% of that obtained using the standard (i.e., 30 min) *ACE* soil protein method. Thus, we chose a 15-min extraction period as optimal. Using a 15-min extraction, *MACE* soil protein concentrations for the individual soils were comparable to those obtained using the standard *ACE* method—again, aligning close to the 1:1 line (Figure 9.3). And again, there was no difference ($P = 0.564$) between soil protein concentrations determined using the 15-min *MACE* protocol and the standard 30-min *ACE* protocol.

The standard operating procedure for the *ACE* soil protein method required approximately 90 min (including the time to heat up and cool down the autoclave) to complete the extraction process. By comparison, microwave-assisted extraction was completed in only 40 min. Thus, *MACE* soil protein extraction can be performed in less than half the time it takes for the conventional autoclave method without affecting protein recovery. Thus, using the 15-min *MACE* protocol with a high capacity (i.e., 24 position) carousel and the BCA protein quantification method we can now comfortably process up to 120 samples per 8-h day.

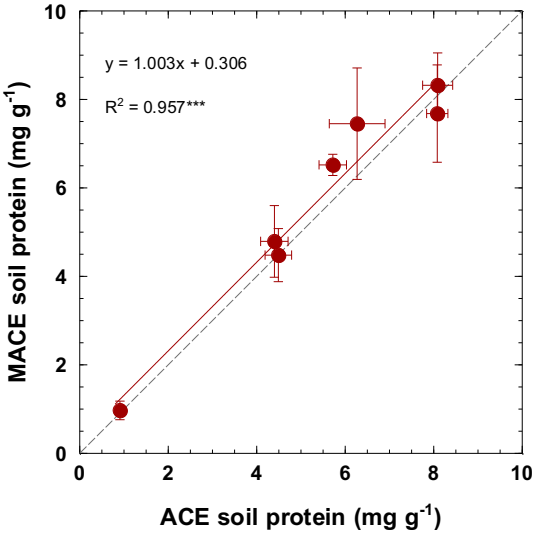

Figure 9.1. *Soil protein concentrations determined using the 30-min autoclaved citrate extractable (ACE) protein method (x-axis) and the 30-min microwave-assisted citrate extractable (MACE) protein method (y-axis). Protein was extracted from 1.0 g of soil in 8 ml of sodium citrate at pH 7 and protein concentrations were determined using the bicinchoninic acid (BCA) protein assay. Mean values (n = 6) are shown as red circles (l); the error bars represent one standard deviation from the mean. The dashed gray line shows the 1:1 relationship; the solid red line is the regression line. *** indicates significance at the P = 0.001 level of probability.*

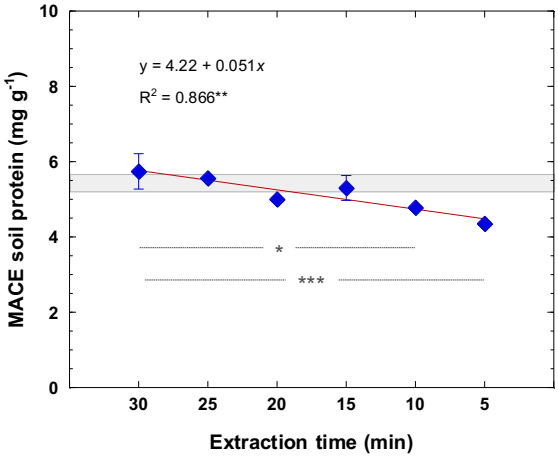

Figure 9.2. *Effect of extraction time on microwave-assisted citrate extractable (MACE) soil protein. The blue diamonds (♦) represent the means ± standard errors for replicate (n = 6) determinations of MACE protein averaged across soils. The gray box represents the mean ± s.d. for the ACE soil protein. The solid red line (—) is the regression line for the relationship between soil protein concentration and extraction time. *, **, *** indicates significance at the P = 0.05, 0.01, and 0.001 levels of probability.*

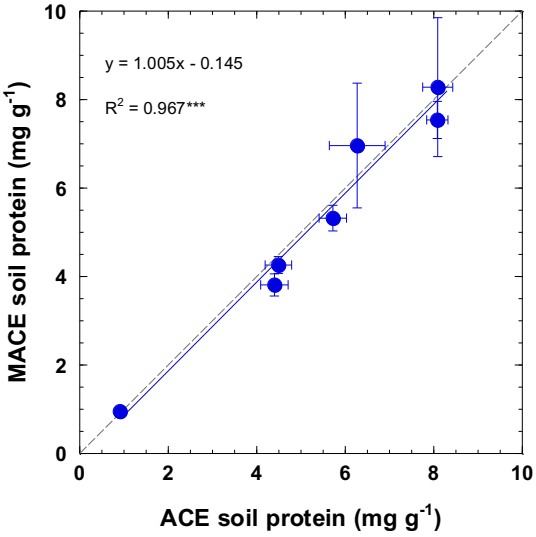

Figure 9.3. *Comparison of soil protein concentrations determined using the 30-min autoclaved citrate extractable (ACE) protein analysis (x-axis) and the 15-min microwave-assisted citrate extractable (MACE) protein analysis (y-axis). Protein was extracted from 1.0 g of soil in 8 ml of sodium citrate at pH 7 and protein concentrations were determined using the bicinchoninic acid (BCA) protein assay. Mean values (n = 6) are shown as blue circles (l); the error bars represent one standard deviation from the mean. The dashed gray line shows the 1:1 relationship; the solid red line is the regression line. *** indicates significance at the P = 0.001 level of probability.*

Soil protein represents an important soil N pool that is sensitive to management (Geisseler et al., 2019; Vasconcellos et al., 2016), is closely associated with arbuscular mycorrhizal fungi, aggregate stability, soil carbon and nitrogen cycling

(Agnihotri et al., 2022; Singh et al., 2017, 2013), and is an important indicator of overall soil health (Hurisso et al., 2018). Thus, increasing the ease for soil analysis labs to determine the soil protein pool more rapidly is key to filling a gap in routine soil testing and soil health interpretation. The *MACE* method developed here fills that gap by providing a faster and more accessible method for determining soil protein.

To the best of our knowledge, this work represents the first use of microwave-assisted citrate extraction (*MACE*) for the determination of soil protein. A 15-min microwave-assisted extraction time yielded soil protein concentrations comparable to those obtained using the standard *ACE* method. Moreover, the data also suggest that by changing the extraction time different fractions of the soil protein pool may be extracted, which may present new opportunities to examine this and other soil organic matter pools in greater detail.

9.2 Assessment of soil protein concentration in Saskatchewan soils

The soils collected from agricultural fields (n = 55) across the province in fall 2018 exhibited levels of *ACE* protein N and total N that varied greatly both across and within the major soil zones (Figs. 9.4 & 9.5). In general, *ACE* protein N in the upper 30 cm of the soil profile decreased in the order: Grey soils > Black soils > Brown soils > Dark Brown soils, while total N decreased in the order: Black soils > Dark Brown soils > Grey soils > Brown soils. The different patterns reflect differences in the fraction of TN accounted for as *ACE* protein; i.e., *ACE* protein N accounted for about 73% of TN in the Grey soils but only 42 to 49% of TN in the Black, Brown, and Dark Brown soils. Differences in *ACE* protein N were generally greatest in the top 15 cm of the soil profiles ($P < 0.001$) with concentrations being greatest in the Grey soils, intermediate in the Black soils, and least in the brown and Dark Brown soils. Conversely, differences in *ACE* protein concentrations among the soil zones were not significant ($P = 0.289$) in the 30–60 cm depth increment.

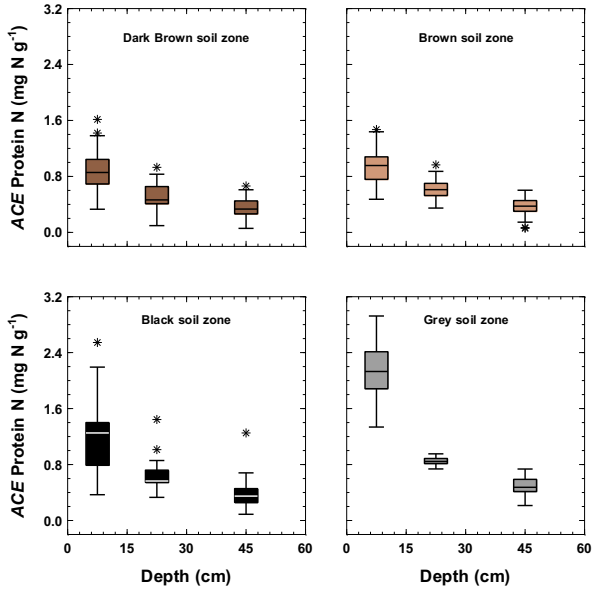

Figure 9.4. *Distribution of ACE protein nitrogen (ACE-PN) in the Dark Brown (n = 21), Brown (n = 17), Black (n = 13) and Grey (n = 4) soil zones in Saskatchewan. Soils were collected in fall (post-harvest) 2018. Statistical outliers are marked with an asterisk (*). Data are plotted at the mid-point of the sampling depth (e.g., at 7.5 cm for the 0–15 cm depth increment.*

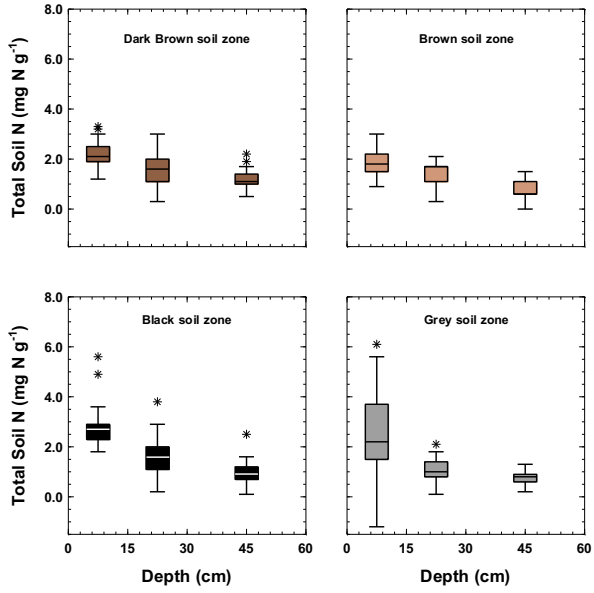

Figure 9.5. *Distribution of soil total nitrogen (TN) in the Dark Brown (n = 21), Brown (n = 17), Black (n = 13) and Grey (n = 4) soil zones in Saskatchewan. Soils were collected in fall (post-harvest) 2018. Statistical outliers are marked with an asterisk (*). Data are plotted at the mid-point of the sampling depth (e.g., at 7.5 cm for the 0–15 cm depth increment.*

Not surprisingly, there were strong correlations ($P \leq 0.001$) between soil protein N and both total soil N and soil organic C (Fig. 9.6A & 9.6B, respectively). Conversely, there was only a very weak correlation between soil protein N and soil inorganic N (Fig. 9.6C: $P = 0.009$). These results were not surprising given that inorganic N represents a snapshot of the

available N at the time the sample was collected, while the soil protein N is thought to represent a reserve pool of potentially mineralizable N. However, we found that there was no significant correlation (P = 0.117) between soil protein N and potentially mineralizable N in the surface (0–15 cm) soils (Fig. 9.6D). Moreover, whereas soil protein N accounted for a significant fraction (*ca*. 50%) of the total N in these soils, PMN accounted for only about 1% of total soil N. Together, these data indicate that—at least over the short-term—only a small fraction (1.5–2.3%) of the N extracted as ACE soil protein is biologically available.

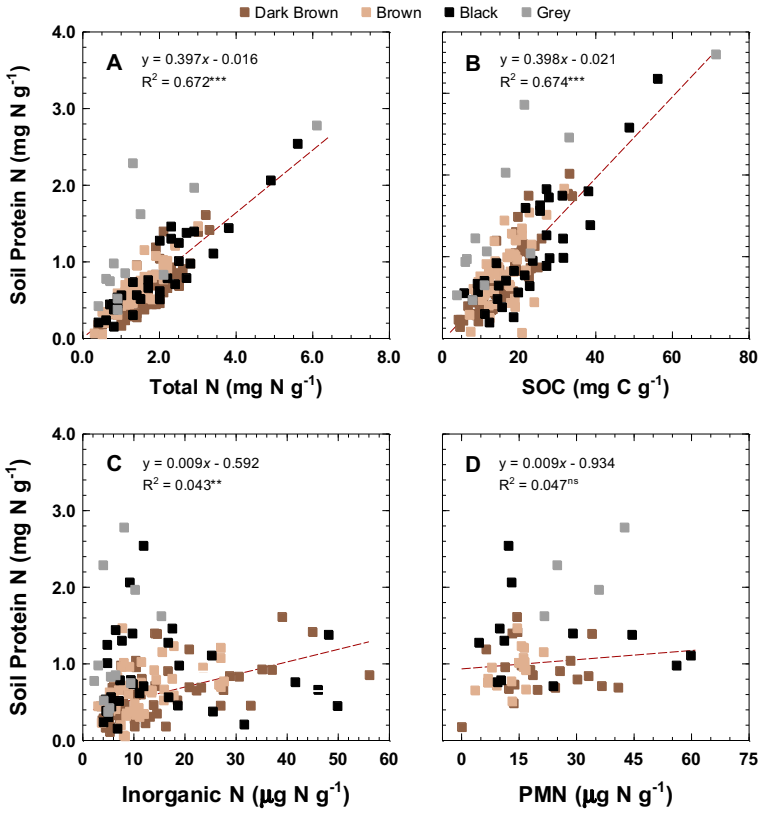

Figure 9.6. *Relationships between ACE soil protein N and (A) total soil N, (B) soil organic C (SOC), (C) inorganic soil N and (D) potentially mineralizable soil N (PMN). Note: PMN was determined only for the surface (0–15 cm) soils.*

9.3 Fertilizer-N response studies

2019 Field season: Fertilizer response curves for wheat and canola grown at the AgriARM sites in 2019 are shown in Figure 9.7. Wheat yields varied significantly among the sites and averaged across N treatments decreased in the order: WARC > ICDC > NARF > WCA ≈ IHARF > CLC (Table 9.1). Fertilizer response decreased as the N rate increased, with the largest response (*ca*. 39%) accompanying the first increment of applied N (i.e., the 0.5× the soil test recommendation). Increasing the N rate to the soil test recommendation (i.e., the 1× rate) resulted in an average yield increase of 13%; however, further increases in the N rate failed to produce a significant (P = 0.05) increase in yield.

Canola yields (averaged across N treatments) also were greatest at the WARC site and decreased in the order: WARC > ICDC > ECRF ≈ CLC ≈ IHARF > WCA > NARF (Table 9.1). In terms of fertilizer response, the largest response in canola yield also occurred with the first increment of applied N (i.e., the 0.5× the soil test recommendation), though the percent increase was much greater (*ca.* 72%) at the NARF and IHARF sites than at the other sites—where the average yield increase

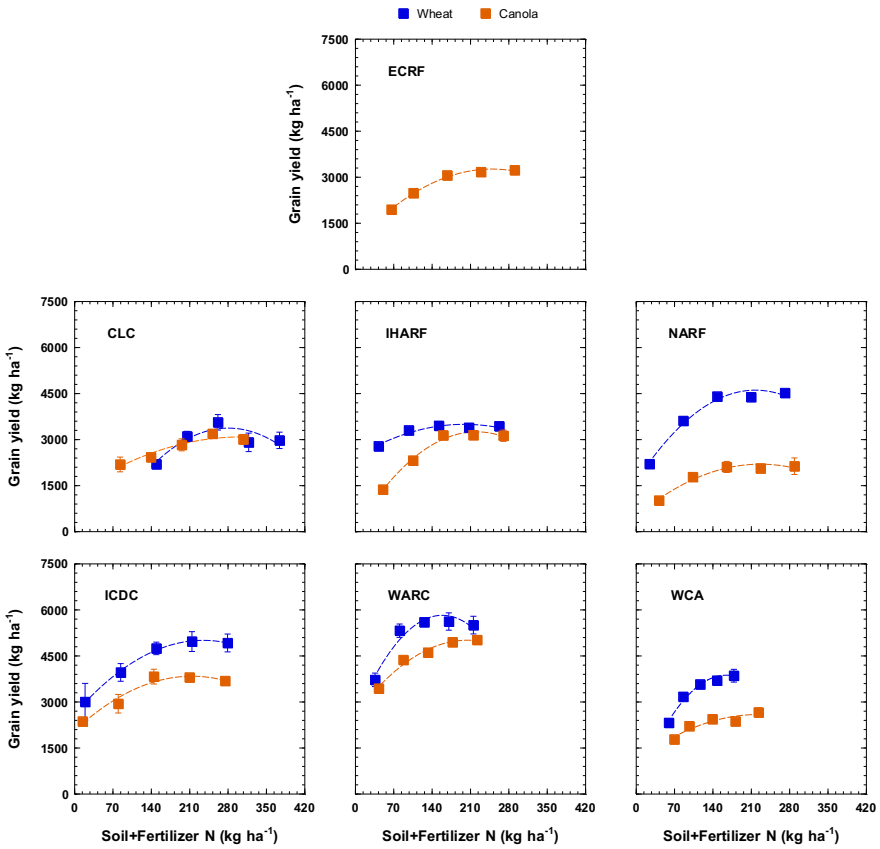

Figure 9.7. *Nitrogen response curves for wheat and canola grown at the Agri-ARM sites in Prince Albert (CLC), Melfort (NARF), Outlook (ICDC), Indian Head (IHARF), Swift Current (WCA), Scott (WARC), and Yorkton (ECRF) Saskatchewan in 2019. The data points correspond to N applications equivalent to 0-, 0.5-, 1.0-, 1.5-, and 2.0-times the soil test recommendation. Error bars represent the standard error of the mean.*

Table 9.1. Seed yields (Mg grain ha^{-1}) of wheat and canola at the AgriARM sites in 2019.

N-rate[a]	ECRF	CLC	IHARF	NARF	ICDC	WARC	WCA
			Wheat [b]				
0×	nc	2.20 b	2.78 b	2.20 c	3.00 b	3.72 b	2.32 c
0.5×	nc	3.11 ab	3.30 a	3.61 b	3.96 ab	5.09 a	3.17 b
1×	nc	3.56 a	3.45 a	4.41 a	4.76 a	5.66 a	3.58 ab
1.5×	nc	2.91 ab	3.39 a	4.38 a	4.97 a	5.64 a	3.70 ab
2×	nc	2.98 ab	3.44 a	4.52 a	4.92 a	5.50 a	3.86 a
Overall mean[c]	nc	2.95 E	3.27 D	3.83 C	4.32 B	5.19 A	3.32 D
P-value	nc	0.0029	<0.0001	<0.0001	0.0002	0.0003	<0.0001
			Canola [b]				
0×	1.94 c	2.19 c	1.37 c	1.02 b	2.37 c	3.44 c	1.78 b
0.5×	2.48 b	2.43 bc	2.32 b	1.78 a	2.94 bc	4.37 b	2.21 ab
1×	3.06 a	2.83 abc	3.14 a	2.11 a	3.83 a	4.61 ab	2.44 a
1.5×	3.17 a	3.19 a	3.15 a	2.06 a	3.80 a	4.95 a	2.37 a
2×	3.23 a	3.01 ab	3.13 a	2.13 a	3.68 ab	5.02 a	2.66 a
Overall mean[c]	*2.78 C*	*2.73 C*	*2.62 C*	*1.82 E*	*3.32 B*	*4.48 A*	*2.29 D*
P-value	*<0.0001*	*0.0094*	*<0.0001*	*0.0019*	*0.0002*	*<0.0001*	*<0.0001*

[a] N-rate is presented as a multiple of the soil test recommendation (STR); e.g., 0× = non-fertilized control, 0.5× = one-half the STR, 1× = STR, etc...

[b] Within columns, means followed by the same lower-case letter are not significantly different (Tukey's HSD, $P \leq 0.05$).

[c] Within the row, means followed by the same upper-case letter are not significantly different (Tukey's HSD, $P \leq 0.05$).

was *ca.* 23%. Increasing the N rate to the soil test recommendation (i.e., the 1× rate) resulted in an average yield increase of 32% at the IHARF and ICDC sites, compared to only about 15% at the other sites. As was the case with wheat, there was no significant (P = 0.05) yield increase at N rates greater than the soil test recommendation (i.e., the 1× rate).

2020 Field season: Fertilizer response curves for wheat and canola grown at the AgriARM sites in 2020 are shown in Figure 9.8, with crop yields summarized in Table 9.2. In general, yield response to N fertilizer was much more variable in 2020 than in 2019—with N-rate having no significant effect on wheat or canola yields at the CLC and WCA sites. Likewise, there was no N-rate effect on canola yields at the NARF site. At the ECRF and IHARF sites the first N-increment (i.e., the 0.5× rate) produced a small but significant increase in yield—with no further increase in yield at the higher N-rates. Moreover, N-rates greater than the 0.5× rate produced a significant increase in wheat yields only at the ICDC and WARC sites, and only at the WARC site did N-rates greater than the 1× rate produce a significant yield increase in wheat.

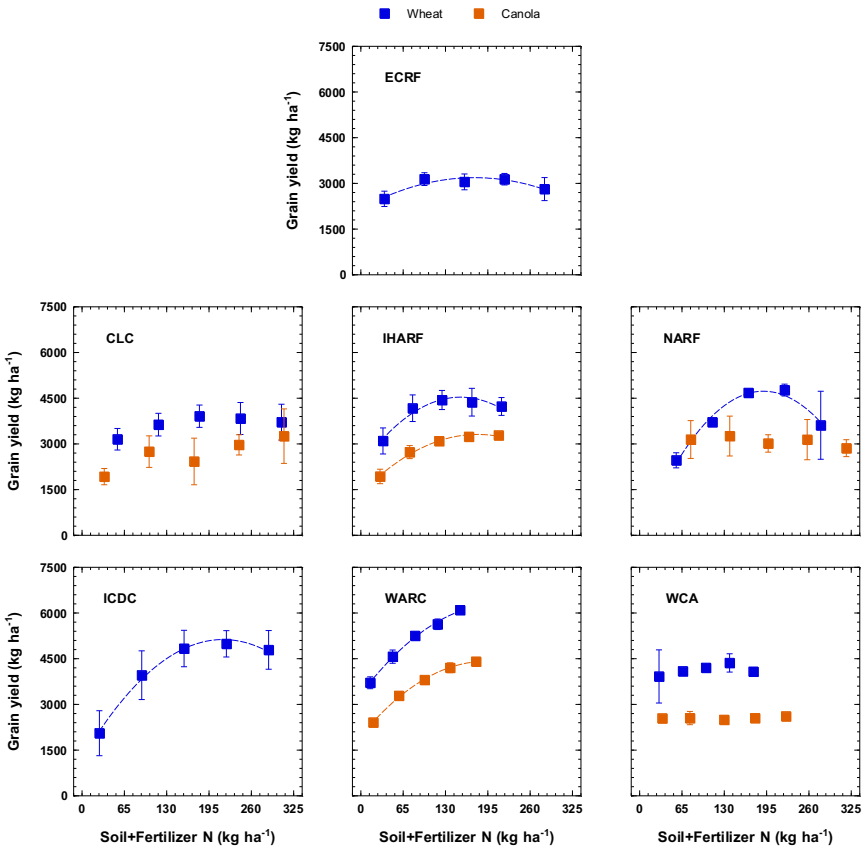

Figure 9.8. *Nitrogen response curves for wheat and canola grown at the Agri-ARM sites in Prince Albert (CLC), Melfort (NARF), Outlook (ICDC), Indian Head (IHARF), Swift Current (WCA), Scott (WARC), and Yorkton (ECRF) Saskatchewan in 2020. The data points correspond to N applications equivalent to 0-, 0.5-, 1.0-, 1.5-, and 2.0-times the soil test recommendation. Error bars represent the standard error of the mean.*

In general, wheat yields averaged across N treatments were greatest at the WARC site (5.05 Mg ha^{-1}; i.e., *ca*. 75 bu acre^{-1}) where they were 1.73-times greater than at the lowest yielding site (i.e., ECRF: 2.93 Mg ha^{-1}; i.e., *ca*. 43 bu acre^{-1}). Wheat yields at the other sites averaged 3.97 ± 0.20 Mg ha^{-1} (*ca*. 59 bu acre^{-1}) and did not differ significantly. Yield response of canola to the application of fertilizer-N also was greatest at the WARC site (Figure 9.2), and though canola yields at the CLC tended to increase with increasing N rate, differences between N-rates were not significant (Table 9.2). Averaged across N treatments, canola yields were greatest at Scott (averaging 3.62 Mg ha^{-1}; i.e., *ca*. 75 bu acre^{-1}) and lowest at Swift Current (WCA: 2.55 Mg ha^{-1}; i.e., *ca*. 45 bu acre^{-1}). Average yields at the other sites did not differ significantly (i.e., by < 15%)—averaging about 2.87 ± 0.21 Mg ha^{-1} (*ca*. 51 bu acre^{-1}).

Table 9.2. Seed yields (Mg grain ha^{-1}) of wheat and canola at the AgriARM sites in 2020.

N-rate[a]	ECRF	CLC	IHARF	NARF	ICDC	WARC	WCA
				Wheat [b]			
0×	2.49 b	3.15	3.10 b	2.46 c	2.05 c	3.71 e	3.92
0.5×	3.14 a	3.63	4.17 a	3.71 ab	3.96 b	4.57 d	4.09
1×	3.05 a	3.91	4.44 a	4.68 ab	4.88 a	5.25 c	4.20
1.5×	3.14 a	3.83	4.37 a	4.77 a	4.99 a	5.63 b	4.36
2×	2.81 ab	3.71	4.23 a	3.61 b	4.79 a	6.10 a	4.08
Overall mean[c]	*2.93 C*	*3.67 B*	*4.06 B*	*3.84 B*	*4.12 B*	*5.05 A*	*4.13 B*
P-value	*0.0034*	*0.1761*	<0.0001	0.0002	<0.0001	<0.0001c	0.6395
				Canola [b]			
0×	nc	1.93	1.93 c	3.14	nc	2.41 e	2.54
0.5×	nc	2.75	2.73 b	3.26	nc	3.28 d	2.55
1×	nc	2.42	3.09 ab	3.01	nc	3.80 c	2.49
1.5×	nc	2.97	3.24 a	3.14	nc	4.20 b	2.55
2×	nc	3.25	3.28 a	2.86	nc	4.40 a	2.61
Overall mean[c]	*nc*	*2.66 CD*	*2.85 BC*	*3.08 B*	*nc*	*3.62 A*	*2.55 D*
P-value	*nc*	*0.0745*	*<0.0001*	*0.8236*	*nc*	*<0.0001*	*0.8632*

[a] N-rate is presented as a multiple of the soil test recommendation (STR); e.g., 0× = non-fertilized control, 0.5× = one-half the STR, 1× = STR, etc...

[b] Within columns, means followed by the same lower-case letter are not significantly different (Tukey's HSD, $P \leq 0.05$).

[c] Within the row, means followed by the same upper-case letter are not significantly different (Tukey's HSD, $P \leq 0.05$).

Averaged across N-rates, wheat yields were greater (by an average of 24%) in 2020 than in 2019 at the CLC, WCA, and IHARF sites. Conversely, at the NARF, ICDC, and WARC sites, inter-annual differences in yield were less than 3%. It is interesting to note that sites with the largest inter-annual differences in wheat yield were those at which there was no significant N-response—or only a very small N-response—indicating that something other than N was the limiting factor.

For canola, the strongest yield responses were observed at the WARC and IHARF sites in both 2020 and 2019. At the same time, inter-annual variations were greatest at the WCA site where there was no yield response for canola in 2020 compared to a moderate yield response in 2019.

Crops growing in the plots that did not receive fertilizer N (i.e., the 0N treatment) were dependent upon the amount of soil-N available during the growing season. Consequently, there was a reasonable expectation that crop yield would correlate with the amount of potentially mineralizable N (PMN)[3] and/or ACE protein in the soil. However, data analysis found a weak negative correlation between crop yield and ACE protein (r = -0.294; $P = 0.082$; see Figure A1). At the same time, there correlation (r = 0.xxx; $P = 0.658$) between crop yield and soil inorganic N (NO_3-N + NH_4-N). There was, however, a moderate positive correlation (r = 0.668; $P < 0.001$) between ACE protein and residual soil NH_4-N after harvest (Figure A3).

10. Conclusions and Recommendations.

Our data indicate that the soil protein pool extracted using the ACE/MACE methodology is not an appropriate measure of the N that potentially becomes biologically available during the season. We hypothesize that these methods extract an inordinately large fraction (*ca*. 40–50%) of the total soil organic N pool, thus obscuring any useful measure of potentially biologically available N. Consequently, we conclude that ACE/MACE measures of soil protein are not suitable as a short-term (seasonal) N availability index

Response of wheat and canola to fertilizer N varied between years and sites. For example, in 2019 wheat and canola grown at all sites exhibited a significant N response, whereas in 2020 three of the five sites where canola was grown, and two of

[3] Note: soils collected in 2019 were not analysed for potentially mineralized N due to the campus shutdown following declaration of the COVID-19 pandemic.

the six sites where wheat was grown failed to produce a significant N response. In both years, the strongest N response by wheat occurred at Scott (WARC), Outlook (ICDC), and Melfort (NARF). Moreover, except for the Scott site, the optimum yield generally occurred at N rates between 0.5× and 1.0× the soil test recommendation.

In general, trends suggested that the largest response in canola and wheat yield occurred with the first increment of applied N (i.e., the 0.5× the soil test recommendation) with further increases occurring at the 1× soil test recommendation rate. Statistically significant yield increases often were generally not observed above the 1× rate, suggesting that current soil test recommendations provide an acceptable measure of biologically available N, with the understanding that the vagaries of weather strongly influence N responses. Importantly, soil tests identified sites less likely to respond significantly to fertilizer N due to relatively high levels of initial soil inorganic N [e.g., CLC (wheat and canola) and NARF (canola) in 2020].

11. Is there a need to conduct follow up research?

There remains a need to develop a rapid chemical test to determine biologically available soil N. Although soil protein is a known source of biologically available N, the ACE/MACE methodology used to determine soil protein appears to measure a much larger fraction of the organic N pool than actually contributes to potentially mineralizable N. Moreover, the strong correlation between total soil N and soil protein N suggests that simply measuring total soil N is as useful as determining soil protein N using the current methods. However, because soil protein is mineralized in a step-wise manner, a method that determines the release of N in a similar step-wise manner may help identify those components of the soil protein pool that are released quickly and contribute to the biologically available N. We hypothesize that pre-treatment of the soil sample with a peptidase may simulate the early release of available N from the soil and may provide a better measure of potentially available N than the current methods. Further research is required to test this hypothesis and develop appropriate methods.

12. Patents/ IP generated/ commercialized products:

None.

13. List technology transfer activities:

Farrell, R.E. (2021). Interview by Geoff Geddes: “Fertilizer research offers food for thought”. Commissioned by the Alberta Wheat Commission, Feb. 4, 2021. Spotlight on AWC-funded Research. https://www.albertawheatbarley.com/alberta-wheat/research/projects.

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14. List any industry contributions or support received.

Funding for this project was provided by Western Grains Research Foundation (WGRF 1813), the Saskatchewan Wheat Development Commission (SWDC 171025-62), the Saskatchewan Canola Development Commission (SCDC CARP ADF2017-288), and the Alberta Wheat Commission (AWC 18AWC56A).

To provide added value to this project, we collaborated with Dr. Kate Congreves to share samples collected from across the province. Dr. Congreves research focused on developing a soil health assessment protocol (SHAP) for Saskatchewan producers (ADF Project No. 20170151) and, as part of this project, she participated in the development of the microwave-assisted citrate extractable (MACE) soil protein test as a rapid indicator of soil health.

15. Acknowledgements.

We acknowledge that this research was carried out on Treaty Six Territory and the Homeland of the Métis. We pay our respect to the First Nation and Métis ancestors of this place and reaffirm our relationship with one another. This research would not have been possible without the cooperation and assistance of staff at the AgriARM sites in in Prince Albert, Melfort, Outlook, Indian Head, Swift Current, Scott, and Yorkton. In addition, the technical support of Mark Cooke during collection of the soil samples and Athena Wu during development of the MACE soil protein test is gratefully acknowledged.

16. Appendices.

16.1 Literature Cited

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16.2 Supplemental Material

Table A1. Crop management information for the ***wheat*** plots at Prince Albert (CLC), Melfort (NARF) and Outlook (ICDC) in 2019.

	CLC	NARF	ICDC
Location	Prince Albert	Melfort	Outlook
Plot size	13.5 m^2	16.2 m^2	12 m^2
Preceeding crop	canola	canola	canola
	Management		
Seeding			
Variety	Certified Cardale	CDC Titanium	AAC Brandon
Date (dd-mm-yyyy)	31-05-2019	14-05-2019	13-05-2019
Target rate	300 plants m^{-2}	250 plants m^{-2}	300 plants m^{-2}
Row spacing	10 in	12 in	10in
Harvest Operations			
Desiccant	None	None	None
Harvest (dd-mm-yyyy)	07-10-2019 (5 of 6 rows)	09-10-2019 (centre 5 rows)	24-09-2019 (centre 6 rows)
	Crop inputs		
Fertilizer (total; kg N ha^{-1}) [a]	112.4	123.6	130
Monoammonium phosphate (MAP; kg N ha^{-1})	- - -	- - -	6.4
Ammonium sulfate (AS; kg N ha^{-1})	- - -	- - -	None
Urea (1× rate; kg N ha^{-1})	112.4	123.6	124
Chemical			
Seed treatment [date: dd-mm-yyyy]	None	Vibrance Quattro	None
Herbicide (pre-emergent) [date: dd-mm-yyyy]	None	None	CleanStart 08-05-2019
Herbicide (in-crop) [date: dd-mm-yyyy]	Axel Extreme (0.5 L ac^{-1}) + MCPA Ester 600 (0.37 L ac^{-1}) 19-06-2019	Axial (0.50 L ac^{-1}) 27-06-2019 Prestige XC A & B (0.13 L ac^{-1}; 0.6 L ac^{-1}) 04-07-2019	Badge II & Simplicity 10-06-2019
Fungicide [date: dd-mm-yyyy]	Pivot 418EC (60 mL ac^{-1}) 19-06-2019	None	Caramba 18-07-2019
Insecticide [date: dd-mm-yyyy]	None	None	None

[a] The crop also received an in-crop foliar application of Kinetic Copron (5% Cu + 2.5% S) applied at a rate of 0.5 L ac^{-1}.

Table A1 cont'd. Crop management information for the ***wheat*** plots at Indian Head (IHARF), Swift Current (WCA), Scott (WARC), and Yorkton (ECRF) in 2019.

	IHARF	WCA	WARC
Location	Indian Head	Swift Current	Scott
Plot size	39 m^{-2}	7.6 m^{-2}	12.2 m^{-2}
Preceeding crop	canola	wheat	canola
	Management		
Seeding			
Variety	Landmark VB	CDC Adament	AAC Brandon
Date (dd-mm-yyyy)	14-05-2019	13-05-2019	14-05-2019
Target rate	325 plants m^{-2}	25 plants ft^{-2}	300 plants m^{-2}
Row spacing	12 in	8.25 in	10 in
Harvest Operations			
Desiccant	Roundup Transorb HC (0.67 L ac^{-1}) 29-08-2019	None	Heat LQ (42.8 mL ac^{-1}) + Roundup 540 (0.67 L ac^{-1}) + Merge (0.2 L ac^{-1}) 06-09-2019
Harvest (dd-mm-yyyy)	07-09-2019 (centre 5 rows)	28-08-2019 (centre 7 rows)	17-09-2019 (all 6 rows)
	Crop inputs		
Fertilizer (total; kg N ha^{-1})	129.5	61.7	92.0
Monoammonium phosphate (MAP; kg N ha^{-1})	6.4	4.2	3.2
Ammonium sulfate (AS; kg N ha^{-1})	13.1	- - -	8.8
Urea (1× rate; kg N ha^{-1})	110	57.5	80.0
Chemical			
Herbicide (pre-emergent) [date: dd-mm-yyyy]	Roundup Weathermax 540 (0.67 L ac^{-1}) 16-05-2019	None	Glyphosate 540 (1 L ac^{-1}) + AIM (35 mL ac^{-1}) 19-05-2019
Herbicide (in-crop) [date: dd-mm-yyyy]	OcTTain XL (0.45 L ac^{-1}) + Simplicity GoDRI (28 g ac^{-1}) 17-06-2019	Varro (0.20 L ac^{-1}) + OcTTain XL (0.45 L ac^{-1}) 04-06-2019	Axial (0.5 L ac^{-1}) + Buctril (0.4 L ac^{-1}) 26-06-2019
Fungicide [date: dd-mm-yyyy]	Prosaro XT (0.325 L ac^{-1}) 11-07-2019	Acapella (0.25 L ac^{-1}) 17-06-2019	None
Insecticide [date: dd-mm-yyyy]	None	None	None

Table A2. Crop management information for the ***canola*** plots at the Prince Albert (CLC), Melfort (NARF) and Outlook (ICDC) sites in 2019.

	CLC	NARF	ICDC
Location	Prince Albert	Melfort	Outlook
Plot size	13.5 m^2	16.2 m^2	12 m^2
Preceeding crop	wheat	wheat	wheat
	Management		
Seeding			
Variety	PV760TM [a]	L233P [b]	L252
Date (dd-mm-yyyy)		14-05-2019	13-05-2019
Target rate	100 plants m^{-2}	120 plants m^{-2}	200 plants m^{-2}
Row spacing	10 in	12 in	10 in
Harvest Operations			
Desiccant	Reglone 06-09-2019	None	None
Harvest (dd-mm-yyyy)	24-09-2019 (5 of 6 rows)	08-10-2019 (centre 5 rows)	24-09-2019 (centre 6 rows)
	Crop inputs		
Fertilizer (total; kg N ha^{-1})	168.5	123.6	130
Monoammonium phosphate (MAP; kg N ha^{-1})	- - -	- - -	5.3
Ammonium sulfate (AS; kg N ha^{-1})	- - -	- - -	- - -
Urea (1× rate; kg N ha^{-1})	168.5	123.6	125
Chemical			
Herbicide (pre-emergent) [date: dd-mm-yyyy]	None	Koril 235 (61 mL ac^{-1}) + Glyphosate 540 (0.5 L ac^{-1}) 16-05-2019	Commercially pre-treated
Herbicide (in-crop) [date: dd-mm-yyyy]	Centurion (77 mL ac^{-1}) 12-06-2019	Liberty 150 (1.35 L ac^{-1}) + Centurion (77 mL ac^{-1}) 27-06-2019	CleanStart 08-05-2019
Fungicide [date: dd-mm-yyyy]	None	Acapella (325 mL ac^{-1}) 12-07-2019	Liberty & Centurion & Amigo 17-06-2019
Insecticide [date: dd-mm-yyyy]	Lumiderm™ (seed applied)	None	None

[a] Certified seed with a pre-applied seed treatment consisting of the insecticide thiamethoxam (Helix®); the fungicides difenoconazole, metalaxyl-M and S-isomer, fludioxonil and sedaxane (Vibrance®); and the insecticide 5-Chloro-2-methyl-2H-isothiazol-3-one (Lumiderm™).

[a] Certified seed with a pre-applied seed treatment consisting of xxxxxx.

Table A2 cont'd. Crop management information for the ***canola*** plots at the Indian Head (IHARF), Swift Current (WCA), Scott (WARC), and Yorkton (ECRF) sites in 2019.

	IHARF	WCA	WARC	ECRF
Location	Indian Head	Swift Current	Scott	Yorkton
Plot size	39 m^{-2}	7.6 m^{-2}	12.2 m^{-2}	30.7 m^{-2}
Preceeding crop	canaryseed	wheat	wheat	wheat
	Management			
Seeding				
Variety	L233P	L233P	L255PC	DKTF92SC
Date (dd-mm-yyyy)	12-05-2019	13-05-2019	22-05-2019	16-05-2019
Target rate	6.2 kg ha^{-1} (125 seeds m^{-2})	6.0 lbs ac^{-1}	125 seeds m^{-2}	5.35
Row spacing	12 in	8.25 in	10 in	12 in
Harvest Operations				
Desiccant	Roundup Transorb (0.67 L ac^{-1}) 29-08-2019	None	Reglone ION (0.83 L ac^{-1}) 18-09-2019	Reglone 05-09-2019
Harvest (dd-mm-yyyy)	16-09-2019 (centre 5 rows)	20-09-2019 (centre 7 rows)	06-10-2019 (xx of xx rows)	13-09-2019 (centre 4 rows)
	Crop inputs			
Fertilizer (total; kg N ha^{-1})	129.5	84.1	92.0	120
Monoammonium phosphate (MAP; kg N ha^{-1})	6.4	11.8	3.2	5
Ammonium sulfate (AS; kg N ha^{-1})	13.1	2.8	8.8	13
Urea (1× rate; kg N ha^{-1})	110	69.5	80.0	102
Chemical				
Herbicide (pre-emergent) [date: dd-mm-yyyy]	Roundup Weathermax 540 (0.67 L ac^{-1}) 12-05-2019	None	Glyphosate 540 (1 L ac^{-1}) + AIM (35 mL ac^{-1}) 19-05-2019	None
Herbicide (in-crop) [date: dd-mm-yyyy]	Liberty 150SN (1.35 L ac^{-1}) + Centurion (0.05 L ac^{-1}) + Amigo (0.5%) 19-06-2019	Liberty 150SN (1.35 L ac^{-1}) + Centurion (0.07 L ac^{-1}) + Amigo (0.5%) 04-06-2019 Liberty 150SN (1.35 L ac^{-1}) 18-06-2019	Liberty (1.62 L ac^{-1}) + Centurion (75 mL ac^{-1}) + Amigo (0.5%) 26-06-2019	Roundup Transorb (0.33 L ac^{-1}) 10-06-2019 Roundup Transorb (0.35 L ac^{-1}) 18-06-2019 Centurion (0.15 L ac^{-1}) + Amigo 26-06-2019
Fungicide [date: dd-mm-yyyy]	Lance (140 g ac^{-1}) + Headline 250EC (0.132 L ac^{-1}) 09-07-2019	None	Priaxor (180 mL ac^{-1}) 15-07-2019	Lance (140 g ac^{-1}) 09-07-2019
Insecticide [date: dd-mm-yyyy]	Matador (33.5 mL ac^{-1}) 12-06-2019	None	None	None

Table A3. Crop management for the ***wheat*** plots at the AgriARM sites in Indian Head (IHARF), Swift Current (WCA), Scott (WARC), and Yorkton (ECRF) in 2020.

	IHARF	WCA	WARC	ECRF
Location	Indian Head	Swift Current	Scott	Yorkton
Plot size	39.6 m^2	17.5 m^2	12.2 m^2	30.7 m^2
Preceeding crop	Canola	Durum	Canola	Canola
		Management		
Seeding				
Variety	CDC Alida VB	CDC Adament	AAC Brandon	Redberry Wheat
Date (dd-mm-yyyy)	14-05-2020	07-05-2020	12-05-2020	16-05-2020
Target rate	139 kg ha^{-1} (325 seeds m^2)	25-27 plants ft^{-2}	300 plants m^{-2} (112 lbs ac^{-1})	126 lb/ac
Row spacing	12 in	8.25 in	10 in	12 in
Harvest Operations				
Desiccant	Roundup Transorb (0.67 L ac^1) [19-08-2020]	None	Heat LQ (59 mL ac^{-1}) + Glyphosate (1 L ac^{-1}) + Merge (0.02 L ac^{-1}) [31-08-2020]	Roundup Transorb (0.66L ac^{-1}) [05-08-2020]
Harvest (dd-mm-yyyy)	[27-08-2020] (centre 5 rows)	[24-08-2020] (7 centre rows)	[18-09-2020] (all 6 rows)	[08-17-2020] (centre 4 rows)
		Crop inputs		
Fertilizer (total; kg N ha^{-1})	110	77.2	70.9	130.4
Monoammonium phosphate (MAP; kg N ha^{-1})	6.4	4.7	1.9	7.2
Ammonium sulfate (AS; kg N ha^{-1})	13	- - -	- - -	- - -
Urea (1× rate; kg N ha^{-1})	90.6	72.5	69.0	123.2
Chemical				
Seed treatment [date: dd-mm-yyyy]	None	Cruiser Vibrance Quattro (325 mL per 100 kg seed) [07-05-2020]	None	None
Herbicide (pre-emergent) [date: dd-mm-yyyy]	Roundup Transorb HC (0.67 L ac^{-1}) [14-05-2020]	Glyphosate (1 L ac^{-1}) + Aim (0.49 L ac^{-1}) [04-05-2020]	Glyphosate 540 (1 L ac^{-1}) + AIM (35 mL ac^{-1}) [09-05-2020]	None
Herbicide (in-crop) [date: dd-mm-yyyy]	Octain (0.67 L ac^{-1}) + Simplicity GoDRI (28 g ac^{-1}) [15-06-2020]	Achieve (0.2 L ac^{-1}) + Buctril M (0.4 L ac^{-1}) + turbocharge (0.5 L per 100 L) [29-05-2020]	Axial (0.5 L ac^{-1}) + Infinity (0.33 L ac^{-1}) [15-06-2020]	Prestige XL (0.85 L ac^{-1}) [02-06-2020] Simplicity 30 OD (0.2 L ac^{-1})+ Agral 90 (0.25 L ac^{-1}) [08-06-2020]
Fungicide [date: dd-mm-yyyy]	Prosaro XTR (325 mL ac^{-1}) [11-07-2020]	None	Caramba (0.2 L ac^{-1}) [16-07-2020]	Caramba (0.4 L ac^{-1}) [02-07-2020]
Insecticide [date: dd-mm-yyyy]	None	None	None	None

Table A3 cont'd. Crop management for the ***wheat*** plots at the AgriARM sites in Prince Albert (CLC), Melfort (NARF), and Outlook (ICDC) in 2020.

	CLC	NARF	ICDC
Location	Prince Albert	Melfort	Outlook
Plot size	10.6 m^2	14 m^2	12 m^2
Preceeding crop	Canola	Canola	Canola
		Management	
Seeding			
Variety	Certified Cameron	AAC Brandon	AAC Brandon
Date (dd-mm-yyyy)	29-05-2020	23-05-2020	25/05/2020
Target rate	300 plants m^{-2}	250 plants m^{-2}	300 plants m^{-2}
Row spacing	10 in	12 in	10 in
Harvest Operations			
Desiccant	None	None	None
Harvest (dd-mm-yyyy)	[23-09-2020] (All rows)	[22-09-2020] (centre 5 rows)	[17-09-2020] (centre 6 rows)
		Crop inputs	
Fertilizer (total; kg N ha^{-1})	135.5	120	136.4
Monoammonium phosphate (MAP; kg N ha^{-1})	9.5	9	6.4
Ammonium sulfate (AS; kg N ha^{-1})	- - -	- - -	- - -
Urea (1× rate; kg N ha^{-1})	126	111	130
Chemical			
Seed treatment [date: dd-mm-yyyy]	None	Raxil Pro [23-05-2020]	Cruiser® Vibrance® Quatro (325 mL per 100 kg seed) [20-05-2020]
Herbicide (pre-emergent) [date: dd-mm-yyyy]	None	Heat LQ (59 mL ac^{-1})+ Glyphosate540 (0.67 L ac^{-1}) [24-05-2020]	CleanStart (360 g ae ac^{-1}) [14-05-2020]
Herbicide (in-crop) [date: dd-mm-yyyy]	Infinity (0.34 L ac^{-1}) [10-06-2020]	Prestige XC (0.13 L ac^{-1} of A+0.6L ac^{-1} of B) [23-06-2020] Axial (0.5 L ac^{-1}) [03-07-2020]	Infinity (0.33 L ac^{-1}) [09-06-2020] Simplicity (28 g ac^{-1}) [10-06-2020]
Fungicide [date: dd-mm-yyyy]	Twinline (0.564 L ac^{-1}) [21-07-2020]	Carambe (400 mL ac^{-1}) [24-07-2020]	None
Insecticide [date: dd-mm-yyyy]	None	None	none

Table A4. Crop management for the ***canola*** plots at the AgriARM sites at Indian Head (IHARF), Swift Current (WCA), and Scott (WARC) in 2020.

	IHARF	WCA	WARC
Location	Indian Head	Swift Current	Scott
Plot size	39.6 m^2	17.5 m^2	12.2 m^2
Preceeding crop	Oat	Durum	Wheat
		Management	
Seeding			
Variety	InVigor L345PC	Dekalb Liberty Link 21SC	L255PC
Date (dd-mm-yyyy)	14-05-2020	07-05-2020	18-05-2020
Target rate	5.9 kg ha^{-1} (125 seeds m^{-2})	8-11 plants ft^{-2} (6lbs ac^{-1})	110 seeds m^{-2} (5 lbs ac^{-1})
Row spacing	12 in	8.25 in	10 in
Harvest Operations			
Desiccant	Roundup Transorb HC (0.67 L ac^{-1}) [29-08-2020]	None	Reglone ION (0.83 L ac^{-1}) [04-09-2020]
Harvest (dd-mm-yyyy)	10-09-2019 (centre 5 rows)	27-08-2020 (7 centre rows)	14-09-2020 (All 6 rows)
		Crop inputs	
Fertilizer (total; kg N ha^{-1})	110	105	84.4
Monoammonium phosphate (MAP; kg N ha^{-1})	6.4	5	1.5
Ammonium sulfate (AS; kg N ha^{-1})	13	5	4
Urea (1× rate; kg N ha^{-1})	90.6	95	78.9
Chemical			
Seed treatment [date: dd-mm-yyyy]	Commercially pre-treated	Commercially pre-treated	Commercially pre-treated
Herbicide (pre-emergent) [date: dd-mm-yyyy]	Liberty 150SN (1.6 L ac^{-1})+ Centurion (50 mL ac^{-1})+Amigo (0.5%) [16-06-2020]	Glyphosate (1 L ac^{-1}) + Aim (0.49 L ac^{-1}) [04-05-2020]	Glyphosate 540 (1 L ac^{-1}) + AIM (35 mL ac^{-1}) [14-05-2020]
Herbicide (in-crop) [date: dd-mm-yyyy]	Liberty 150SN (1.35 L ac^{-1}) [02-07-2020]	Liberty 150SN (1.35 L ac^{-1}) + Centurion (0.05 L ac^{-1}) + Amigo (0.5%) [02-06-2020] Liberty 150SN (1.35 L ac^{-1}) + Centurion (0.05 L ac^{-1}) + Amigo (0.5%) [19-06-2020]	Liberty (1.62 L ac^{-1}) + Centurion (75 mL ac^{-1}) + Amigo (0.5 L ac^{-1}) [18-06-2020]
Fungicide [date: dd-mm-yyyy]	Lane (140 g ac^{-1})+ Headline 250 EC (0.132 L ac^{-1}) [15-07-2020]	None	Priaxor (120 mL ac^{-1}) [09-07-2020]
Insecticide [date: dd-mm-yyyy]	None	None	None

Table A4 cont'd. Crop management for the ***canola*** plots at the AgriARM sites in Prince Albert (CLC) and Melfort (NARF) in 2020.

	CLC	NARF
Location	Prince Albert	Melfort
Plot size	10.6 m^2	14 m^2
Preceeding crop	Barley	Wheat
	Management	
Seeding		
Variety	PV 540	L233P
Date (dd-mm-yyyy)	01-06-2020	23-05-2020
Target rate	120 plants m^{-2}	120 seeds m^{-2}
Row spacing	10 in	12 in
Harvest Operations		
Desiccant	None	Glyphosate540 (0.67L ac^{-1}) [08-09-2020]
Harvest (dd-mm-yyyy)	30-09-2020 (All rows)	23-09-2020 (centre 5 rows)
	Crop inputs	
Fertilizer (total; kg N ha^{-1})	143	147.4
Monoammonium phosphate (MAP; kg N ha^{-1})	5	10.7
Ammonium sulfate (AS; kg N ha^{-1})	- - -	16.7
Urea (1× rate; kg N ha^{-1})	138	120
Chemical		
Seed treatment [date: dd-mm-yyyy]	Commercially pre-treated	Commercially pre-treated
Herbicide (pre-emergent) [date: dd-mm-yyyy]	Glyphsate (0.67 L ac^{-1}) [05-06-2020]	None
Herbicide (in-crop) [date: dd-mm-yyyy]	None	Liberty (1.35 L ac^{-1}) + Centurion (75 mL ac^{-1}) + Amigo (0.5 L ac^{-1}) [16-06-2020]
Fungicide [date: dd-mm-yyyy]	Priaxor (0.180 mL ac^{-1}) [17-07-2020]	Acapella (485 mL ac^{-1}) [16-07-2020]
Insecticide [date: dd-mm-yyyy]	None	None

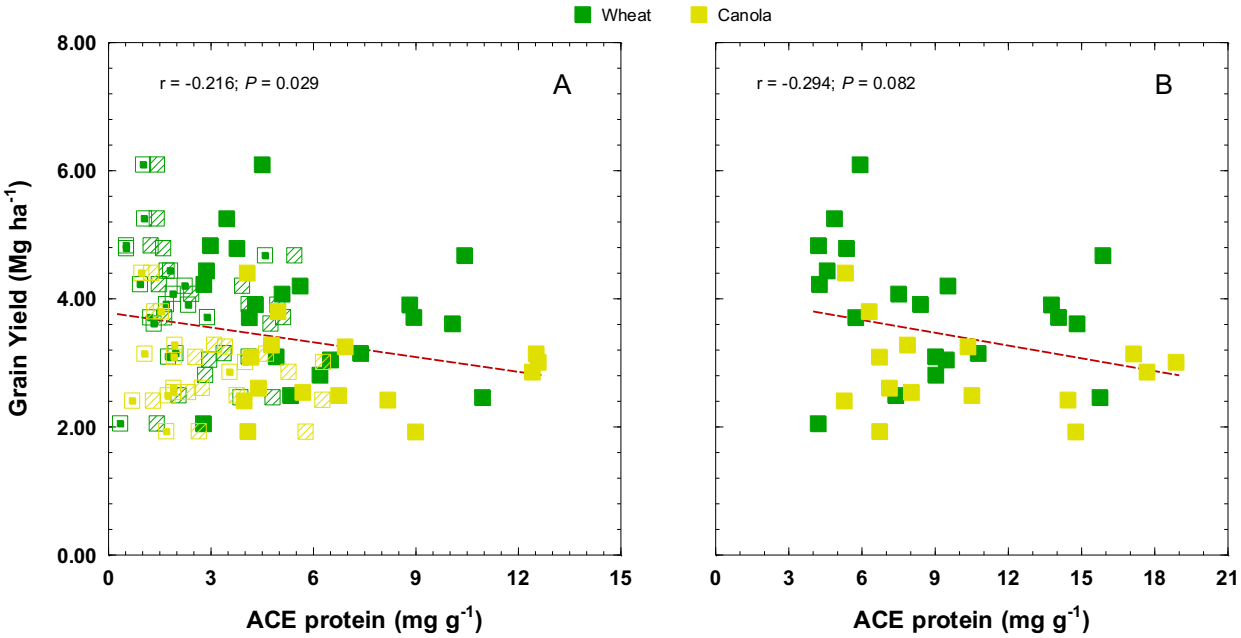

Figure A1. Relationship between grain yield and total ACE protein in the 0–15 cm (■), 15–30 cm (▨), and 30–60 cm (▣) depth increments (A) and total ACE protein in the upper 60 cm of the soil profile (B).

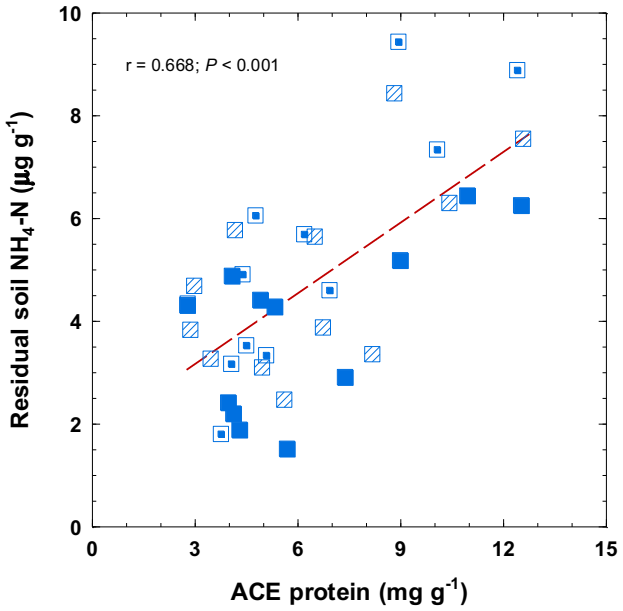

Figure A2. Relationship between residual soil ammonium (NH_4^+-N) and ACE protein. Samples were collected from plots receiving N applications equal to 0× (■), 1× (▨), and 2× (▣) the soil test recommendation.