Use of Soil Protein Pools as Indicators of Soil Nitrogen Mineralization Potential

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Autoclaved-citrate extractable (ACE) soil protein is included in some soil health assessments as a biological indicator. Furthermore, soil protein contents may be related to the ability of a soil to make nitrogen (N) available for plants by mineralization. The main objective of this study was to evaluate the correlation between ACE protein and potential net N mineralization in undisturbed soil cores from 57 fields in California under annual crops. Total N in the soils ranged from 0.65 to 12.5 g kg^{-1}, and the sites represented eight Soil Taxonomy orders. Soil ACE protein concentrations ranged from 1.0 to 45.2 g kg^{-1} soil. Although the correlation between ACE protein and potential net N mineralization was positive, ACE protein explained only 21% of the variability in potential net N mineralization across all sites, which was less than total N. Under the assumption that proteins contain 16% N, ACE protein-N accounted for 28% of total N across all sites. However, in some soils with a high total N content, ACE protein accounted for up to 67% of total N. Because autoclaving is expected to denature some proteins, these values seem very high and are likely caused by the interference of coextracted humic substances. Our results do not suggest that ACE protein is a better predictor of potential net N mineralization than total soil N, which may be at least partly due to an apparent interference of coextracted humic substances with the protein assay.

Abbreviations: ACE, autoclaved-citrate extractable; BCA, bicinchoninic acid; BRSP, Bradford-reactive soil protein; CASH, Comprehensive Assessment of Soil Health; DI, deionized; FDA, fluorescein diacetate; PON, particulate organic nitrogen; POXC, permanganate oxidizable carbon; SOM, soil organic matter.

Core Ideas

- **ACE protein concentrations ranged from 1.0 to 45.2 g kg^{-1} soil.**
- **ACE protein and N mineralization were positively correlated (r = 0.46).**
- **The correlation was weaker than between N mineralization and total soil N.**
- **Co-extracted humic substances appear to interfere with the protein assay.**

Soil is the foundation of agricultural production, and unsustainable practices have led to its degradation worldwide (FAO, 2011). The increasing awareness of the importance of soil has led to an interest in evaluating its quality and health (Doran and Zeiss, 2000). Soil health assessments are generally based on different soil chemical, physical, and biological properties. Nitrogen (N) mineralization is a crucial process contributing to soil fertility and soil health. In agricultural systems, N is often the limiting nutrient. Plants take up N from the soil solution mainly in the forms of nitrate and ammonium. Although the mineral N pool is generally small compared with the total soil N pool (Bronson, 2008), its turnover rate can be fast (e.g., Osterholz et al., 2017). Microbial mineralization of organic forms of N constantly replenishes mineral N in solution. Reliable estimates of the amount of N that is mineralized from organic material and becomes potentially plant available allow adjusting fertilizer rates to ensure high yields while minimizing losses. Potential N mineralization rates are often determined by incubating soils in the laboratory for several weeks or months (Jarvis et al., 1996; Stanford and Smith, 1972). However, to be adopted by soil test laboratories, soil tests need to be rapid and inexpensive. Despite decades of research, accurate estimation of N mineralization remains a challenge (Vigil et al., 2002). This is especially true for

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California, where a large number of crops are grown on a wide variety of soil types (Johnston, 2003; Zinke and Delwiche, 1983). Previous work with agricultural soils from California's Central Valley did not identify tests or soil properties with strong enough correlations with the N mineralization potential to be used commercially (Geisseler, 2005; Miller et al., 2019; Wade et al., 2016).

Proteinaceous materials comprise a large pool of organic soil N, with amino acid–N accounting for 30 to 45% of total N in soil hydrolysates (Stevenson, 1982). Other important pools include amino sugars and heterocyclic N compounds (Schulten and Schnitzer, 1998). Depolymerization of proteins by extracellular enzymes into short peptides and free amino acids is considered to be rate limiting for organic matter breakdown and production of inorganic N (Schimel and Bennett, 2004). Soil protein content, therefore, may be related to the ability of a soil to make N available for plants through mineralization (Moebius-Clune et al., 2016). The Comprehensive Assessment of Soil Health (CASH), which has been offered by the Cornell University Soil Health Laboratory for the past decade, includes a soil protein analysis as a biological indicator (Fine et al., 2017; Moebius-Clune et al., 2016). The analytical method includes the extraction of proteins from soil by autoclaving a sample with 20 mM sodium citrate at pH 7.0 for 30 min (Hurisso et al., 2018; Moebius-Clune et al., 2016) followed by protein quantification. This extraction method is based on research by Wright and Upadhyaya (1996), who developed it to extract glomalin from soil. With the exception of glomalin, the harsh extraction procedure is assumed to destroy the vast majority of proteins (Rosier et al., 2006). In their study, Wright and Upadhyaya (1996) quantified proteins in the extracts using the Bradford assay (Bradford, 1976).

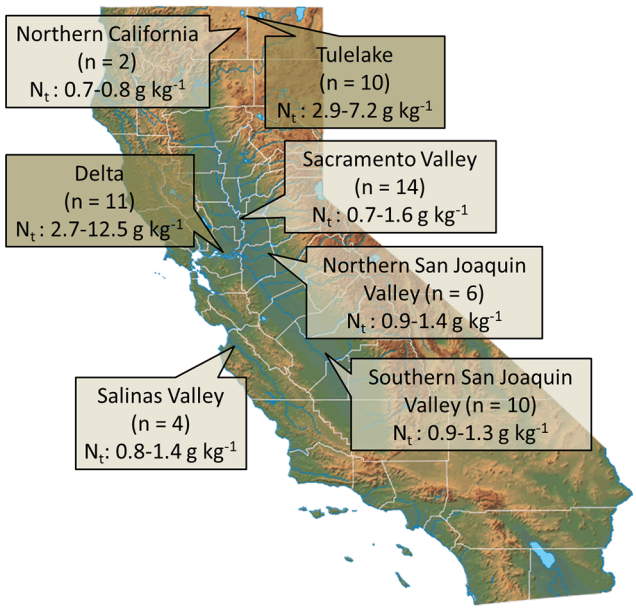

Fig. 1. Location of the sites included in this study. Darker-colored text boxes represent regions with soils with a high total N (N_t) content; lighter colored text boxes are sites with a low N_t content. The N_t values represent the range for the sites sampled in each region. The number of sites (n) sampled in each region is also shown.

This extraction procedure has been used widely by the scientific community; however, different terms were used for the extracted protein fraction. Initially referred to as "easily extractable glomalin," the fraction was renamed "glomalin-related soil fractions" to acknowledge the fact that the link between glomalin and the extracted proteins was not clearly established (Rillig, 2004). Another term used in the literature for the same fraction is "easily extractable Bradford-reactive soil protein" (BRSP) because the Bradford assay for protein quantification is nonspecific for a particular protein (Rillig, 2004). In fact, several studies demonstrated that the procedure extracts not only glomalin but also protein from a wide range of organic sources (Hurisso et al., 2018; Rosier et al., 2006). Most recently, Hurisso et al. (2018) proposed to refer to the extracted protein pool as autoclaved-citrate extractable (ACE) soil protein or simply soil protein. Although the extraction procedure has not changed, the CASH uses the bicinchoninic acid (BCA) assay (Smith et al., 1985) for protein quantification instead of the Bradford assay. In this paper, we use the term BRSP when referring to studies where the protein concentration in autoclaved 20 mM sodium citrate extracts was quantified using the Bradford assay and use the term ACE protein when quantified by the BCA assay.

Soil protein extraction is a potentially informative method for inclusion in soil health assessments for a number of reasons. Several studies reported positive correlations with soil aggregation (Fine et al., 2017; Rillig et al., 2002; Wright and Upadhyaya, 1996; Wright et al., 1999). The BRSP fraction has also been found to be sensitive to land use (Halvorson and Gonzalez, 2006) and crop management (Wright et al., 1999). However, the relationship between ACE protein and potential net N mineralization has not yet been evaluated (Hurisso et al., 2018). Furthermore, the ACE method coextracts humic, lipid, and inorganic substances from soil (Gillespie et al., 2011). This may be problematic because the BCA assay has been found to overestimate protein concentration in the presence of humic substances (Roberts and Jones, 2008).

Proteins in soil solution, which are readily available, may be a better predictor of N mineralization than more recalcitrant proteins within soil organic matter (SOM) (Roberts and Jones, 2008), including those extracted as ACE proteins. However, studies with extractable organic N, which contains soluble proteins, have found that extractable organic N is temporally variable, its quantity depending strongly on sampling time (Ros et al., 2009).

The objective of the study was to evaluate the correlation between ACE protein, soluble protein, and potential net N mineralization from SOM for 57 soils from fields in California under annual crops to determine whether these protein pools could be used as indicators for potential net N mineralization. The samples represent a wide variety of crops and soil properties. We hypothesize that ACE protein is a good predictor of the N mineralization potential in these soils.

MATERIALS AND METHODS

Site Description

A total of 57 field sites under annual row crops located in the northern half of California were included in this study (Fig. 1).

Because the objective of the study was to determine potential net N mineralization from SOM, fields sites with no recent history (at least 3 yr) of cover crops or application of manure or compost were selected. Soils from the Sacramento–San Joaquin Delta and Tulelake basin had total N contents ranging from 2.7 to 12.5 g kg^{-1} (Table 1). The sites included from these two regions are located on former wetlands drained for agriculture (Adam et al., 1989; Drexler et al., 2009) and were predominantly classified as Mollisols and Histosols, including one Andisol, according to USDA Soil Taxonomy (Soil Survey Staff, 2017).

Soils collected in the other regions (the Sacramento, San Joaquin, and Salinas valleys) as well as two sites in northern California had much lower and less variable total N contents (range, 0.7–1.6 g kg^{-1}). These soils formed on alluvium and were classified as Mollisols, Vertisols, Inceptisols, Entisols, Alfisols, and Aridisols. In total, the soils belonged to eight USDA Soil Taxonomy soil orders. Based on their total N content, which reflects differences in pedogenesis, the soils were grouped into soils with a low and a high total N content (see Data Analyses).

Soil Sampling and Analyses

Undisturbed soil cores (4.5 cm diameter, 15 cm length) were taken pre-plant in spring 2016 and 2017 from the topsoil. The time of sampling was chosen to minimize the effects of recently incorporated crop residues and growing crops on soil analyses. At each site, the field was divided into three random blocks, and two undisturbed soil cores (treated as subsamples) were taken from each block. Representative samples for laboratory analyses, including protein assays, were taken from the same layer with a soil probe from the area surrounding the cores.

Total N was determined by dry combustion on a Costech Elemental Combustion System (ECS 4010) (Nelson and Sommers, 1996). Particulate organic N (PON) was determined using the procedure described by Cambardella and Elliott (1992). The method used to determine permanganate oxidizable carbon (POXC) was based on Weil et al. (2003) with modifications proposed by Culman et al. (2012). The approach used to measure fluorescein diacetate (FDA) hydrolysis was based on Green et al. (2006) and Prosser et al. (2011). The analytical procedures have been described in detail by Miller et al. (2019).

Potential Net N Mineralization Study

Potential net N mineralization was determined in undisturbed cores to minimize the effects of sample handling (i.e., sieving and drying) on N mineralization. For the incubation, cores were adjusted to 60% water filled pore space or 60% water holding capacity, whichever was lower. Soil moisture in the cores was adjusted by injecting deionized (DI) water with a site-port syringe needle. The cores were then covered with punctured plastic wrap and placed in an incubator set to 25°C. Moisture content in the cores was monitored weekly and adjusted with the addition of DI water to maintain an optimal moisture content. After 10 wk, the cores were removed from the sleeves, sieved, and analyzed for ammonium-N and nitrate-N concentrations, which were measured colorimetrically in 0.5 M potassium sulfate extracts (Miller et al., 2019). Potential net N mineralization was calculated by subtracting the initial mineral N (sum of ammonium-N and nitrate-N) in the representative samples from the final mineral N concentration in the cores.

Soil Autoclaved-Citrate Extractable Protein

Soil ACE protein was determined on air-dry samples in accordance with the CASH protocol (Moebius-Clune et al., 2016). The extraction procedure was based on Wright and Upadhyaya (1996) with modifications proposed by Hurisso et al. (2018). Briefly, air-dry soil was sieved to 2 mm, 2 g of soil were weighed into 40-mL glass vials, and 24 mL of a 20 mM sodium citrate solution (pH 7) were added. The vials were then shaken on a reciprocal shaker for 5 min. After shaking, the caps were loosened, and the samples were autoclaved for 30 min at 121°C using the liquid cycle. After autoclaving, the samples were shaken for 3 min on a reciprocal shaker to resuspend the soil particles. An aliquot of the slurry (1.4 mL) was then transferred to a microcentrifuge tube and centrifuged at 10,000 rpm for 5 min. Finally, 0.05 mL of the supernatant was transferred to a microcuvette and mixed with 1 mL of Pierce BCA protein reagent (Thermo Scientific). For samples with a high protein concentration, the sample/reagent ratio was reduced. After letting the color develop for 3 h at 60°C in a ventilated oven, the absorbance was measured at 562 nm on a spectrophotometer. To correct for background color in the solutions, the same amount of supernatant was pipetted into a second microcuvette and mixed with 1 mL of DI water. A sepa-

Table 1. Properties of the soils included. The soils are grouped based on their total N (N_t) content. The soils with a high N_t content are further split based on their geographic origin.

	Soil group				
			High-N_t soils		
Soil property†	All soils	Low-N_t soils	All	Delta	Tulelake
Number of sites	57	36	21	11	10
N mineralization, mg kg^{-1}	35.0 (10.1–127.1)‡	24.8 (10.1–42.7)	52.4 (20.4–127.1)	44.0 (20.4–112.0)	61.7 (22.4–127.1)
Total N, g kg^{-1}	2.73 (0.65–12.48)	1.14 (0.65–1.56)	5.47 (2.71–12.48)	6.22 (2.71–12.48)	4.65 (2.92–7.16)
PON, g kg^{-1}	0.60 (0.05–5.87)	0.15 (0.05–0.30)	1.39 (0.16–5.87)	1.77 (0.16–5.87)	0.96 (0.38–2.07)
POXC, mg kg^{-1}	734 (192–3578)	346 (192–477)	1399 (245–3578)	1966 (775–3578)	776 (245–1564)
FDA hydrolysis, mg kg^{-1} h^{-1}	22.9 (3.4–76.7)	14.6 (3.4–33.6)	37.2 (20.4–76.7)	40.1 (25.1–68.8)	34.0 (20.4–76.7)
Clay, %	37.1 (8.4–68.6)	30.0 (8.4–58.8)	49.3 (32.2–68.6)	43.1 (32.2–61.5)	56.2 (49.4–68.6)
pH	7.27 (5.57–8.08)	7.55 (5.57–8.08)	6.79 (5.9–7.65)	6.54 (5.90–7.27)	7.07 (6.19–7.65)

† FDA, fluorescein diacetate, N mineralization, potential net N mineralization; PON, particulate organic N; POXC, permanganate oxidizable carbon.
‡ Values are means with the range in parentheses.

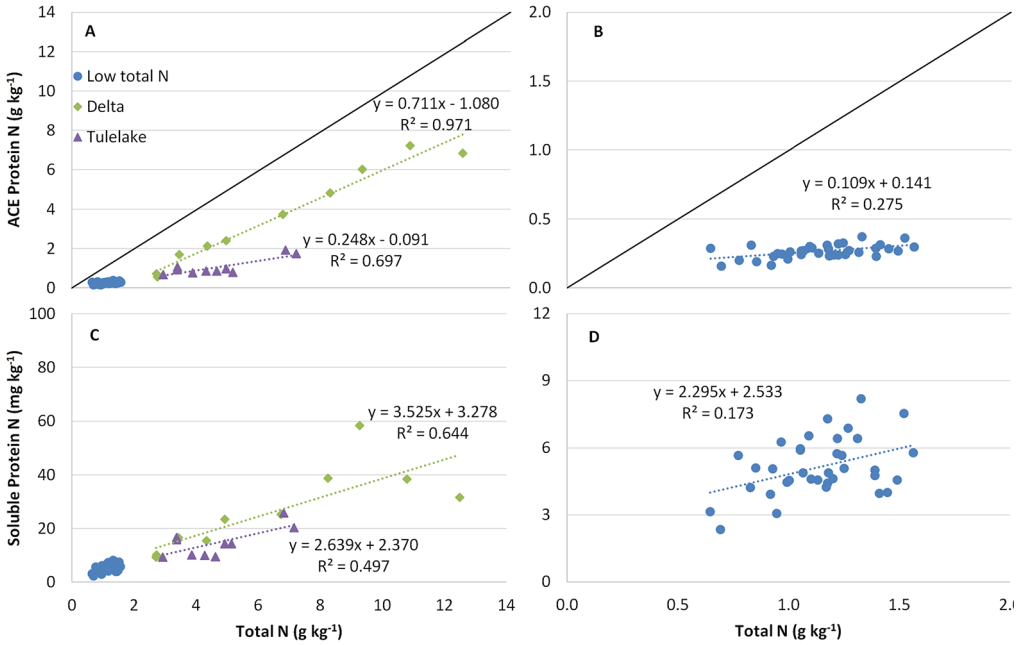

Fig. 2. Relationship between total soil N and autoclaved-citrate extractable (ACE) protein (A and B) and soluble protein (C and D). Panels B and D are enlarged portions of the bottom left corners of panels A and C, respectively, that contain the data from the low total N soils. The diagonal line in panels A and B represents the 1:1 line.

rate standard curve was prepared with bovine serum albumin for each set of samples.

Soluble Protein

Soluble proteins were determined following the procedure described by Weintraub and Schimel (2005). In contrast to their study, air-dry soil was used for the present study. Briefly, air-dry soil was sieved to 2 mm, and 6 g of soil were extracted with 24 mL of a sodium bicarbonate solution (0.1 M) in 40-mL glass vials. The samples were shaken for 1 h on a reciprocal shaker. The slurries were then centrifuged as described above for ACE soil protein. To account for the much lower concentration of protein in solution, 0.2 mL rather than 0.05 mL of sample were mixed with 1 mL of BCA reagent. The samples were then analyzed following the procedure for ACE protein.

Data Analyses

Protein concentration was converted to protein-N concentration using a conversion factor of 6.25, which is based on the assumption that proteins contain 16% N on average (Jones, 1941). Regression and correlation analyses were done using the SAS software system, version 9.4 (SAS Institute, 2013). The analyses were performed on the entire dataset as well as on two groups of soil selected based on total N content, which reflects differences in pedogenesis. The group of soils with a low total N content consisted of soils from the Central Valley (n = 30), from the Salinas Valley (n = 4), and from locations adjacent to the Tulelake basin in northern California (n = 2). Soils with a high total N content included soils from the Tulelake basin (n = 10) and the Sacramento-San Joaquin River Delta (n = 11). Separate analyses were also performed for Delta and Tulelake soils because the results suggested that the interference of co-extracted humic substances on ACE protein determination differed between the soils from these two regions.

RESULTS

Soil Properties and Potential N Mineralization Rates

Total N in the soils ranged from 0.65 to 12.5 g kg^{-1}. Other soil properties related to SOM, such as PON, POXC, and FDA hydrolysis, followed a similar trend. Across all sites, pH ranged from 5.6 to 8.1, and clay content ranged from 8 to 69% (Table 1).

Potential net N mineralization rates in undisturbed soil cores differed considerably across and within regions, ranging from 10 to 127 mg kg^{-1} soil during the 10-wk incubation (Table 1). Variability was especially pronounced across soils from the Delta and Tulelake basin. In contrast, potential net N mineralization was more uniform across soils with a low total N content. However, within this group, the highest potential net N mineralization rate was more than four times greater than the lowest. Across all sites, the coefficient of variation of the three replicates per site averaged 25%.

Soil Autoclaved-Citrate Extractable Protein Concentrations

Soil ACE protein concentrations ranged from 1.0 to 45.2 g kg^{-1} soil. In soils with a low total N content, ACE protein concentrations averaged 1.7 g kg^{-1} soil (range, 1.0–2.3 g kg^{-1} soil). In the soils with a high total N content, ACE protein concentrations ranged from 3.6 to 45.2 g kg^{-1} soil (average, 14.1 g kg^{-1} soil). Across all sites, the coefficient of variation of the three replicates per site averaged 10%.

Under the assumption that proteins contain 16% N, ACE protein-N accounted for 28% of total N across all sites. However, there were large differences among groups (Fig. 2). In soils with a low total N content and in those from the Tulelake basin,

Table 2. Pearson correlation coefficients for the correlation between autoclaved-citrate extractable protein and other soil properties. The soils are grouped based on their total N (N_t) content.

	Group of soils				
			High N_t		
Soil property†	All	Low N_t	All	Delta	Tulelake
N mineralization	0.462***	0.161	0.202	0.548	0.716*
Total N	0.928***	0.527***	0.925***	0.985***	0.836**
PON	0.926***	-0.151	0.888***	0.919***	0.845**
POXC	0.960***	0.345*	0.942***	0.965***	0.803**
FDA hydrolysis	0.759***	0.410*	0.656***	0.848***	0.902***
Clay content	0.208	0.213	−0.562**	−0.346	−0.353
pH	−0.698***	−0.149	−0.763***	-0.831**	−0.440
Soluble protein	0.919***	0.457**	0.878***	0.860***	0.909***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† FDA, fluorescein diacetate; N mineralization, potential net N mineralization; PON, particulate organic N; POXC, permanganate oxidizable carbon.

protein-N accounted for 24% of total N (range, 15–45%). In contrast, in the soils from the Delta, protein-N made up 47% of total N (range, 21–67%). These high values were associated with dark-colored extracts.

Soluble Protein Concentrations

Soluble protein concentrations were much lower than ACE protein concentrations (average, 66.8 mg kg^{-1} soil). Soluble proteins were lower in the soil with a low total N content than in high-N soils (average, 32.2 and 126.0 mg kg^{-1} soil, respectively). Across all sites, the coefficient of variation of the three replicates per site averaged 15%. Soluble proteins accounted for 1.7% of ACE protein and 0.43% of total N across all sites (Fig. 2). The proportion of total soil N in the form of soluble protein N did not differ across groups of soil, with the variability within groups being larger than across groups.

Correlation of Protein Pools with Soil Properties and Potential Net N Mineralization

Across all 57 sites, ACE protein was highly correlated with soil properties related to SOM, namely total N, PON, POXC, and FDA hydrolysis, with correlation coefficients ranging from 0.76 to 0.96. The correlation was stronger in the soils with a high total N content compared with low-N soils (Table 2). Across all sites, the correlation between ACE protein and potential net N mineralization was significant (r = 0.46) (Fig. 3). The correlation, however, was weak for the soils with a low total N content (r = 0.16; p = 0.347).

The ACE protein and soluble protein contents were highly correlated across all sites following a quadratic relationship (Fig. 4). In the soils with a high total N content, the correlation between ACE protein and soluble proteins was also strong (r = 0.88), whereas it only reached 0.46 for the soils with a low total N content.

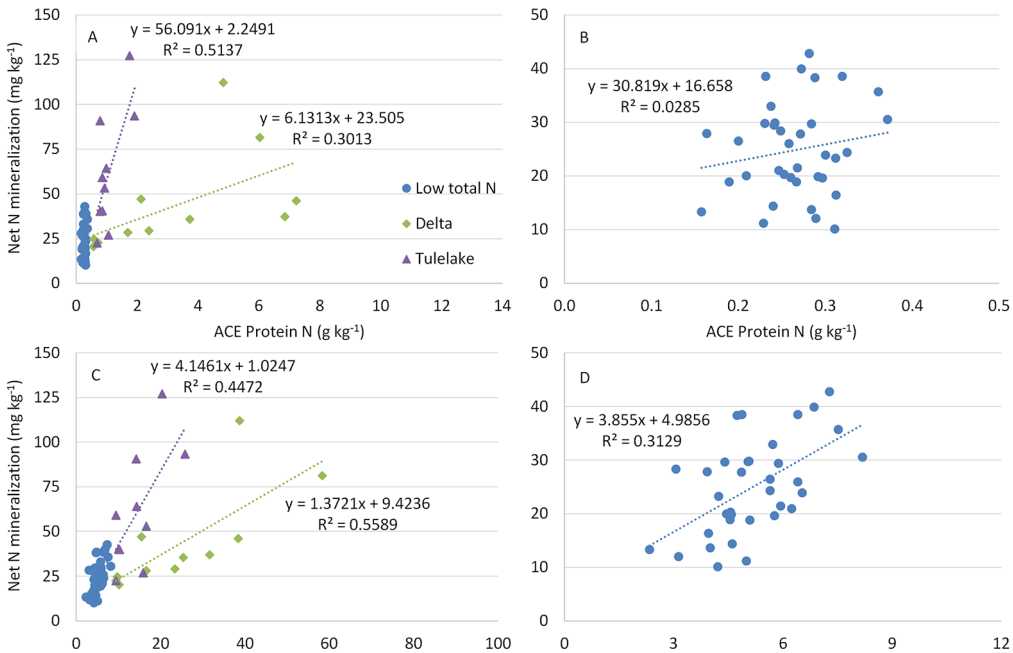

Fig. 3. Relationship between potential net N mineralization and autoclaved-citrate extractable (ACE) protein (A and B) and soluble protein (C and D). Panels B and D are enlarged portions of the bottom left corners of panels A and C, respectively, that contain the data from the low total N soils.

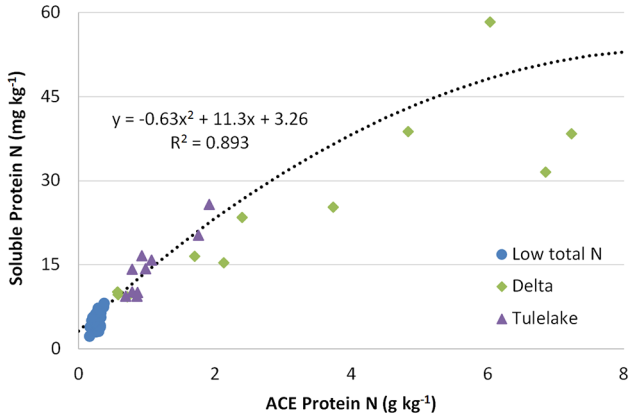

Fig. 4. Relationship between autoclaved-citrate extractable (ACE) protein and soluble protein across all sites.

The correlations between soluble protein and soil properties related to SOM were similar as described for ACE protein above, with the exception that the correlation was weaker in the soils with a low total N content (Table 3). In contrast, soluble protein content showed a stronger correlation with potential net N mineralization than ACE protein across all 57 sites (r = 0.64).

Both protein pools were negatively correlated with pH; however, this may be due to the fact that soil with a high total N content had lower pH values than soils with a low total N content (Table 1).

DISCUSSION

Soil Autoclaved-Citrate Extractable Protein

The correlation between ACE protein and potential net N mineralization was significantly positive. However, at r = 0.46, ACE protein explained only 21% of the variability in potential net N mineralization across all sites. This is less than for total N (r = 0.65), total C (r = 0.54), or FDA hydrolysis (r = 0.71) and similar to PON (r = 0.48) and POXC (r = 0.49) for the same sites (Miller et al., 2019). Furthermore, there was no significant correlation between ACE protein content and potential net N mineralization when soils with a low and high total N content were analyzed separately (Fig. 3). However, the correlation was better (r = 0.72) when the analysis was performed on the high-N soils from the Tulelake region alone. The ACE protein concentrations in the soils with a high total N content were higher than the highest values in the soils with a low total N content. In contrast, there was considerable overlap in potential net N mineralization rates between these two soil groups. These results do not support our hypothesis that ACE protein is a good predictor of potential net N mineralization.

In the soils with a low total N content, ACE protein concentration averaged 1.7 g kg^{-1}. This concentration is lower than the concentration in soils from the Mid-Atlantic, Midwest, and Northeast regions of the United States, where it averaged 7.7 g kg^{-1} (Fine et al., 2017). Fine et al. (2017) found a close correlation between ACE protein and SOM (r = 0.78). Therefore, the difference in ACE protein is likely due to the lower SOM content of 1.8% in our soils compared with 3.6% in those analyzed by Fine et al. (2017).

Across all sites, ACE protein-N accounted for 28% of total N. However, there were large differences among soil groups (Fig. 2). In the soils with a low total N content and in the high-N soils from the Tulelake basin, ACE protein-N accounted for 24% of total N. This is below the range of studies reviewed by Stevenson (1982), who reported that amino acid-N accounts for 30 to 45% of N in soil hydrolysates. In a more recent review by Rillig et al. (2007), the total hydrolyzable amino acid–N content accounted for 18 to 52% of total soil N (approximate average, 36%). However, amino acid–N in soil hydrolysates cannot be directly compared with ACE protein. First, a fraction of the proteinaceous material in soil is in the form of free amino acids and is not detected by the BCA assay. This fraction is generally small (Stevenson, 1982) and is unlikely to result in large differences between the two methods. Second, and more importantly, the harsh conditions of the extraction procedure, which includes autoclaving, are likely to denature some proteins. For example, Rosier et al. (2006) detected only 41 to 84% of added bovine serum albumin with the Bradford assay. Therefore, the proteins detected by the ACE method represent only a fraction of the total proteins in soil. In our study, ACE protein-N accounted for 28% of total N. Under the assumption that the 36% of total N in the form of hydrolyzable amino acid–N (Rillig et al., 2007) is a reasonable estimate of the soil protein pool, the ACE method detected 78%

Table 3. Pearson correlation coefficients for the correlation between soluble protein and other soil properties. The soils are grouped based on their total N (N_t) content.

	Group of soils				
				High N_t	
Soil property†	All	Low N_t	All	Delta	Tulelake
N mineralization	0.637***	0.559***	0.423	0.748**	0.669*
Total N	0.901***	0.415*	0.808***	0.802**	0.705*
PON	0.803***	0.210	0.691***	0.647*	0.731*
POXC	0.899***	0.336*	0.823***	0.790**	0.816**
FDA hydrolysis	0.870***	0.601***	0.801***	0.969***	0.830**
Clay content	0.313*	0.087	−0.462*	−0.218	−0.484
pH	−0.713***	0.079	−0.758***	−0.850***	−0.390

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† N mineralization, potential net N mineralization; PON, particulate organic N; POXC, permanganate oxidizable carbon; FDA; fluorescein diacetate.

of all protein in soil. Therefore, the results from our soils with a low total N content appear to be at the upper limit of the range that would be expected from analyses of soil hydrolysates.

The fraction of total soil N in the form of ACE protein was much higher in the soils from the Delta, averaging 47% of total N (range, 21–67%). Even though amino acid N may comprise a larger proportion of total N in Histosols (Stevenson, 1982), the higher values seem excessive. A likely explanation for this result is the fact that the ACE method co-extracts other substances from soil. Schindler et al. (2007) found that the nuclear magnetic resonance spectroscopy results of total BRSP closely resembled that of humic acid and concluded that BRSP contains proteinaceous material and a large excess of humic acid. These conclusions are in line with Gillespie et al. (2011), who found purified BRSP to be a rich mixture of proteinaceous, humic, lipid, and inorganic substances. In fact, the high values in Delta soils were associated with dark-colored extracts, which may be due to humic acids. Furthermore, such findings are supported by studies where N concentrations of the extracts were determined. Rillig et al. (2001) reported that partially purified extracted BRSP contained on average 1.1% N, whereas Halvorson and Gonzalez (2006) and Schindler et al. (2007) reported N concentrations of 4.02 and 3.8%, respectively. Because proteins generally contain between 16 and 23% N (Mariotti et al., 2008), the results from studies suggest that only a small proportion of the molecules extracted are proteinaceous material and that humic substances and other molecules with a low N content make up a large proportion of the organic molecules in the extracts.

The BCA assay is based on the reaction of copper ions with peptide bonds (Smith et al., 1985), and nonproteinaceous substances in the extract are not expected to interfere with the quantification of proteins. This does not seem to be the case, however. Roberts and Jones (2008) tested several protein assays for interference by humic substances. Their results revealed that the BCA assay suffered most from interference and overestimated protein concentration in the presence of humic substances. It is therefore likely that the high ACE protein values found in the Delta soils are an artifact caused by the interference of coextracted humic substances. In these soils, the proportion of total N in the form of ACE protein was twice as high as in the other soils, suggesting that the interference may be substantial. Although interference was less pronounced with other protein assays, Roberts and Jones (2008) concluded that none of the assays appeared capable of providing a reliable estimate of total protein content in soil solution.

Soluble Protein

Soluble protein-N accounted for 0.43% of total soil N, with little differences across soil groups. This is within the range reported by Weintraub and Schimel (2005), who found that soluble protein-N accounted for 0.3 to 4.3% of total soil N in arctic tundra soils. In contrast to our study, Weintraub and Schimel (2005) did not air-dry the samples before extraction, which may potentially affect the results.

Proteins contained in solution are readily available for microorganisms and may be a better predictor of N mineralization than more recalcitrant proteins within SOM, which are a component of ACE protein (Roberts and Jones, 2008). Our study found a slightly better correlation of potential net N mineralization with soluble protein than with ACE protein. The better correlation may be due to the high bioavailability of the soluble protein fraction or the lower interference by humic substances. However, the use of soluble protein as a measure of net N mineralization potential has its own challenges. Our results show that the variability across the three replicates from each site was larger for soluble proteins than for ACE protein. Although little data are available on the temporal variability of soluble proteins in agricultural soils, extractable organic N, which contains soluble proteins, has been found to be temporally variable, its quantity depending strongly on sampling time (Ros et al., 2009). In addition, soil drying greatly increases extractable organic N and thus likely soluble protein (Ros et al., 2009). The extent of this increase may vary across soil types. Finally, although the amount of humic substances co-extracted with soluble proteins is likely much smaller than with the ACE method, they may still interfere with the protein assay and affect the results because the protein concentration is also much lower than the ACE protein concentration. However, the quadratic relationship between ACE protein and soluble protein may indicate that soluble protein values are less affected by humic substances. Based on these considerations, soluble proteins cannot be promoted as an alternative to ACE protein without a more thorough investigation.

CONCLUSIONS

Our results do not suggest that ACE protein is a better predictor of potential net N mineralization than total soil N or other soil properties related to SOM. Despite this result, ACE protein may be a useful component of soil health assessments because other studies have found that it is correlated with soil aggregate stability and is sensitive to land use and crop management. However, the apparent interference of humic substances raises the question of whether the method is a reliable measure of a soil protein fraction across soils with different SOM contents.

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REFERENCES

Adam, D.P., A.M. Sarna-Wojcicki, H.J. Rieck, J.P. Bradbury, W.E. Dean, and R.M. Forester. 1989. Tulelake, California: The last 3 million years. Palaeogeogr. Palaeoclimatol. Palaeoecol. 72:89–103. doi:10.1016/0031-0182(89)90134-X

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-pye binding. Anal. Biochem. 72:248–254. doi:10.1016/0003-2697(76)90527-3

Bronson, K.F. 2008. Forms of inorganic nitrogen in soil. In: J.S. Schepers and W.R. Raun, editors, Nitrogen in agricultural systems. Agronomy Monograph 49. ASA, CSSA, SSSA, Madison, WI. p. 31–55. doi:10.2134/agronmonogr49.c2

Cambardella, C.A., and E.T. Elliott. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J.

56:777–783. doi:10.2136/sssaj1992.03615995005600030017x

Culman, S.W., S.S. Snapp, M.A. Freeman, M.E. Schipanski, J. Beniston, R. Lal, et al. 2012. Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. Soil Sci. Soc. Am. J. 76:494–504. doi:10.2136/sssaj2011.0286

Doran, J.W., and M.R. Zeiss. 2000. Soil health and sustainability: Managing the biotic component of soil quality. Appl. Soil Ecol. 15:3–11. doi:10.1016/S0929-1393(00)00067-6

Drexler, J.Z., C.S. de Fontaine, and T.A. Brown. 2009. Peat accretion histories during the past 6000 years in marshes of the Sacramento–San Joaquin Delta, CA, USA. Estuaries Coasts 32:871–892. doi:10.1007/s12237-009-9202-8

FAO. 2011. The state of the world's land and water resources for food and agriculture (SOLAW) managing systems at risk. Rome, Italy and Earthscan, London, UK. http://www.fao.org/docrep/017/i1688e/i1688e.pdf (accessed 12 Dec. 2018).

Fine, A., H. Van Es, and R. Schindelbeck. 2017. Statistics, scoring functions, and regional analysis of a comprehensive soil health database. Soil Sci. Soc. Am. J. 81:589–601. doi:10.2136/sssaj2016.09.0286

Geisseler, D. 2005. Evaluation of soil tests for predicting net nitrogen mineralization and their use as fertilization recommendations for field crops. MS thesis, Univ. of California, Davis.

Gillespie, A.W., R.E. Farrell, F.L. Walley, A.R.S. Ross, P. Leinweber, K.U. Eckhardt, et al. 2011. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids, and humic materials. Soil Biol. Biochem. 43:766–777. doi:10.1016/j.soilbio.2010.12.010

Green, V.S., D.E. Stott, and M. Diack. 2006. Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. Soil Biol. Biochem. 38:693–701. doi:10.1016/j.soilbio.2005.06.020

Halvorson, J.J., and J.M. Gonzalez. 2006. Bradford reactive soil protein in Appalachian soils: Distribution and response to incubation, extraction reagent and tannins. Plant Soil 286:339–356. doi:10.1007/s11104-006-9047-x

Hurisso, T.T., D.J. Moebius-Clune, S.W. Culman, B.N. Moebius-Clune, J.E. Thies, and H.M. van Es. 2018. Soil protein as a rapid soil health indicator of potentially available organic nitrogen. Agric. Environ. Lett. 3:180006.

Jarvis, S.C., E.A. Stockdale, M.A. Shepherd, and D.S. Powlson. 1996. Nitrogen mineralization in temperate agricultural soils: Processes and measurement. Adv. Agron. 57:187–235. doi:10.1016/S0065-2113(08)60925-6

Jones, D.B. 1941. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. USDA, Washington, DC.

Johnston, W.E. 2003. Cross sections of a diverse agriculture: Profiles of California's agricultural production regions and principal commodities. In: J. Siebert, editor, California agriculture: Dimensions and issues. Giannini Foundation of Agricultural Economics, Univ. of California, Berkeley. p. 29–55 https://escholarship.org/uc/item/9145n8m1 (accessed 12 Dec. 2018).

Mariotti, F., D. Tomé, and P. Patureau Mirand. 2008. Converting nitrogen into protein: Beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr. 48:177–184. doi:10.1080/10408390701279749

Miller, K., B.J. Aegerter, N.E. Clark, M. Leinfelder-Miles, E.M. Miyao, R. Smith, R. Wilson, and D. Geisseler. 2019. Relationship between soil properties and nitrogen mineralization in undisturbed soil cores from California agroecosystems. Commun. Soil Sci. Plant Anal. 50(1):1–16. doi:10.1080/00103624.2018.1554668

Moebius-Clune, B.N., D.J. Moebius-Clune, B.K. Gugino, O.J. Idowu, R.R. Schindelbeck, A.J. Ristow, et al. 2016. Comprehensive assessment of soil health: The Cornell framework. 3rd ed. Cornell Univ., Ithaca, NY.

Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In: D.L. Sparks, editor, Methods of soil analysis. Part 3. Chemical methods. SSSA, Madison, WI. p. 971–1010.

Osterholz, W.R., O. Rinot, M. Liebman, and M.J. Castellano. 2017. Can mineralization of soil organic nitrogen meet maize nitrogen demand? Plant Soil 415:73–84. doi:10.1007/s11104-016-3137-1

Prosser, J.A., T.W. Speir, and D.E. Stott. 2011. Soil oxireductases and FDA hydrolysis. In: R.P. Dick, editor, Methods in soil enzymology. SSSA, Madison, WI. p. 103–124. doi:10.2136/sssabookser9.c6

Rillig, M.C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci. 84:355–363. doi:10.4141/S04-003

Rillig, M.C., S.F. Wright, K.A. Nichols, W.F. Schmidt, and M.S. Torn. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant Soil 233:167–177. doi:10.1023/A:1010364221169

Rillig, M.C., B.A. Caldwell, H.A.B. Wösten, and P. Sollins. 2007. Role of proteins in soil carbon and nitrogen storage: Controls on persistence. Biogeochemistry 85:25–44. doi:10.1007/s10533-007-9102-6

Rillig, M.C., S.F. Wright, and V. Eviner. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: Comparing effects of five plant species. Plant Soil 238:325–333. doi:10.1023/A:1014483303813

Roberts, P., and D.L. Jones. 2008. Critical evaluation of methods for determining total protein in soil solution. Soil Biol. Biochem. 40:1485–1495. doi:10.1016/j.soilbio.2008.01.001

Ros, G.H., E. Hoffland, C. van Kessel, and E.J.M. Temminghoff. 2009. Extractable and dissolved soil organic nitrogen– A quantitative assessment. Soil Biol. Biochem. 41:1029–1039. doi:10.1016/j.soilbio.2009.01.011

Rosier, C.L., A.T. Hoye, and M.C. Rillig. 2006. Glomalin-related soil protein: Assessment of current detection and quantification tools. Soil Biol. Biochem. 38:2205–2211. doi:10.1016/j.soilbio.2006.01.021

SAS Institute. 2013. Base SAS® 9.4 Procedures guide: Statistical procedures, 2nd ed. SAS Institute Inc., Cary, NC.

Schimel, J.P., and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85:591–602.

Schindler, F.V., E.J. Mercer, and J.A. Rice. 2007. Chemical characteristics of glomalin-related soil protein (GRSP) extracted from soils of varying organic matter content. Soil Biol. Biochem. 39:320–329. doi:10.1016/j.soilbio.2006.08.017

Schulten, H.R., and M. Schnitzer. 1998. The chemistry of soil organic nitrogen: A review. Biol. Fertil. Soils 26:1–15. doi:10.1007/s003740050335

Smith, P.K., R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, et al. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76–85. doi:10.1016/0003-2697(85)90442-7

Stanford, G., and S.J. Smith. 1972. Nitrogen mineralization potentials of soils. Soil Sci. Soc. Am. J. 36:465–472. doi:10.2136/sssaj1972.03615995003600030029x

Stevenson, F.J. 1982. Organic forms of soil nitrogen. In: F.J. Stevenson, editor, Nitrogen in agricultural soils. ASA, CSSA, SSSA, Madison, WI. p. 67–122.

Soil Survey Staff. 2017. Web soil survey. USDA–NRCS, Washington, DC. https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm (accessed 4 Mar. 2019).

Vigil, M.F., B. Eghball, M.L. Cabrera, B.R. Jakubowski, and J.G. Davis. 2002. Accounting for seasonal nitrogen mineralization: An overview. J. Soil Water Conserv. 57:464–469.

Wade, J., W.R. Horwath, and M.B. Burger. 2016. Integrating soil biological and chemical indices to predict net nitrogen mineralization across California agricultural systems. Soil Sci. Soc. Am. J. 80:1675–1687. doi:10.2136/sssaj2016.07.0228

Weil, R.R., K.R. Islam, M.A. Stine, J.B. Gruver, and S.E. Samson-Liebig. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Altern. Agric. 18:3–17. doi:10.1079/AJAA2003003

Weintraub, M.N., and J.P. Schimel. 2005. Seasonal protein dynamics in Alaskan arctic tundra soils. Soil Biol. Biochem. 37:1469–1475. doi:10.1016/j.soilbio.2005.01.005

Wright, S.F., and A. Upadhyaya. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161:575–586. doi:10.1097/00010694-199609000-00003

Wright, S.F., J.L. Starr, and I.C. Paltineanu. 1999. Changes in aggregate stability and concentration of glomalin during tillage management transition. Soil Sci. Soc. Am. J. 63:1825–1829. doi:10.2136/sssaj1999.6361825x

Zinke, P.J., and C.C. Delwiche. 1983. Soils and climate. In: A.F. Scheuring, editor, A guidebook to California agriculture. Univ. of California Press, Berkeley. p. 41–106.