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Authors: Zhang, Bin, Li, Jingyi, Drury, Craig F., Woodley, Alex L., and Yang, Xueming

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ARTICLE

Effect of crop rotation and cropping history on net nitrogen mineralization dynamics of a clay loam soil

Bin Zhang, Jingyi Li, Craig F. Drury, Alex L. Woodley, and Xueming Yang

Abstract: Estimating soil nitrogen (N) mineralization is critical to balance fertilizer N requirements and their environmental impacts. In this study, net N mineralization was examined in soils under different crop rotations with each phase of the rotation present every year with biologically based incubations in 2011 and 2015. Net N mineralization was significantly different among treatments when the current crop was soybean, and the effect was dependent upon the previous crop and the cropping sequence. In particular, net increases in inorganic N were greater when the previous crop was winter wheat with or without red clover than if it were corn, and greater for the first year of soybean compared with the second year for rotations with two consecutive years of soybean in the 2011 incubation. However, cropping history did not influence net soil N mineralization when the current crop was corn, winter wheat, or winter wheat with red clover. In 2015, the presence of red clover in the rotation increased net N mineralization in all phases of the rotation. These results suggest both current and previous crops should be considered when estimating the N supplying capacity (net mineralization) of the soil. Net mineralizable N was found to be significantly correlated with total amino sugars ($P < 0.001$), glucosamine ($P < 0.001$), and galactosamine ($P = 0.003$), which suggests that amino sugars could be used as an indicator of the N supplying capacity of soil.

Key words: soil nitrogen mineralization, amino sugar, crop sequence, cropping history.

Résumé : On doit absolument estimer la minéralisation de l'azote (N) dans le sol pour équilibrer les besoins d'engrais azoté et leurs répercussions sur l'environnement. Dans le cadre de cette étude, les auteurs ont examiné la minéralisation nette du N dans le sol de divers assolements, les différentes phases de ceux-ci revenant chaque année, avec incubation du sol à deux reprises, en 2011 et 2015. La minéralisation nette du N diffère sensiblement d'un traitement à l'autre quand la culture de l'année est le soja. Cet effet dépend de la culture antérieure et de la succession des cultures. Plus précisément, la hausse nette de N inorganique est plus importante quand la culture antérieure est le blé d'hiver, avec ou sans trèfle rouge, et non le maïs. Elle était aussi plus élevée la première année où l'on avait cultivé le soja que la deuxième, dans les assolements qui comprenaient deux années consécutives de cette culture, lors de l'essai d'incubation réalisé en 2011. Néanmoins, l'historique des cultures n'a aucune influence sur la minéralisation nette du N dans le sol quand la culture actuelle consiste en maïs, en blé d'hiver ou en blé d'hiver avec trèfle rouge. En 2015, la présence de trèfle rouge dans l'assolement a accru la minéralisation nette du N dans toutes les phases de l'assolement. Ces résultats laissent croire qu'on devrait prendre en compte la culture de l'année et la culture antérieure quand on veut estimer la quantité de N que peut procurer le sol (minéralisation nette). La quantité nette de N minéralisable est significativement corrélée avec la concentration totale de sucre aminés ($P < 0,001$), de glucosamine ($P < 0,001$) et de galactosamine ($P = 0,003$), ce qui laisse croire qu'on pourrait se servir des sucres aminés pour se faire une idée de la quantité de N pouvant être fournie par le sol. [Traduit par la Rédaction]

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B. Zhang.* School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing, Jiangsu 210044, People's Republic of China; Harrow Research & Development Centre, Agriculture & Agri-Food Canada, Harrow, Ontario N0R 1G0, Canada.

J. Li. School of Applied Meteorology, Nanjing University of Information Science & Technology, Nanjing, Jiangsu 210044, People's Republic of China.

C.F. Drury and X. Yang. Harrow Research & Development Centre, Agriculture & Agri-Food Canada, Harrow, Ontario N0R 1G0, Canada.

A.L. Woodley.* Harrow Research & Development Centre, Agriculture & Agri-Food Canada, Harrow, Ontario N0R 1G0, Canada; Department of Crop and Soil Sciences, North Carolina State University, Raleigh, NC 27695, USA.

Corresponding author: Craig F. Drury (email: craig.drury@agr.gc.ca).

*These authors contributed equally to this work.

Mots-clés : minéralisation de l'azote du sol, sucres aminés, séquence culturale, historique des cultures.

Introduction

The Haber–Bosch process facilitates the production of nitrogen (N) fertilizers, which significantly increased global crop production to support almost half of the world's population (Erisman et al. 2008). However, a large portion of the N fertilizers can be lost through ammonia volatilization (Pan et al. 2016; Drury et al. 2017; Woodley et al. 2020), nitrate leaching (Sebilo et al. 2013; Drury et al. 2016), and nitrous oxide emissions (Reay et al. 2012; Shcherbak et al. 2014), resulting in a low N use efficiency. These losses cause several negative environmental consequences, which include soil acidification (Guo et al. 2010), eutrophication of inland and marine waters from nitrate (Le Moal et al. 2019), and formation of air pollutants by ammonium and nitrate (An et al. 2019). Therefore, it is essential to adopt strategies to minimize N losses from the agricultural soils, increase N use efficiency, and ultimately achieve environmentally friendly crop production.

One strategy to improve soil quality and minimize environmental losses of nutrients may be to enhance crop diversity with better crop rotations (i.e., three or more crops and if feasible cover crops). It is widely acknowledged that corn (*Zea mays* L.) and soybean (*Glycine max* L.) cropping systems can benefit from introducing cover crops into the rotation as the fall crop could utilize residual soil N and increase N use efficiency (Schomberg et al. 2006; Gentry et al. 2013). In addition, cover crops can provide soil N in a critical uptake window for tobacco (*Nicotiana tabacum* L.), while allowing for partial reduction in applied N (Hahn et al. 2021). Recently, it has been reported that the inclusion of winter wheat (*Triticum aestivum* L.) into a rotation also improved N use efficiency of corn- and soybean-based cropping systems (Gaudin et al. 2015). These results can be largely attributed to an increase in plant-derived soil N, and thus, the N supplying capacity of soils (Gaudin et al. 2015). Indeed, crop rotation has been shown to strongly influence soil N mineralization (Verloop et al. 2014; Hahn et al. 2021). However, the influence of cropping history in a rotation on soil N mineralization and the N supplying capacity of soils is largely unknown. This knowledge will help improve N recommendations for crop rotations, which has largely been ignored.

To accurately quantify the N supplying capacity of soil during crop growth, several chemical and biological methods have been recommended (Zebarth et al. 2009). Early methods included the quantification and modeling of potentially mineralizable N under optimal incubation conditions (Stanford and Smith 1972), determination of net inorganic N accumulation with laboratory incubations (Wang et al. 2001), calculation of the N uptake by crops using field or greenhouse experiments (Fox and Piekielek 1984), as well as estimation of soil inorganic N contents either before planting (Roth and Fox 1990) or before top dressing (Meisinger et al. 1992). In more recent years, scientists extracted specific soil organic N fractions and correlated them with soil mineralizable N; however, the validation was largely dependent upon chemical extractions and their concentrations, as well as extraction time and temperature (Ros et al. 2011). A substantial proportion of soil inorganic N can also be immobilized into organic forms by microbes following N fertilizer application to soil (He et al. 2011). As the microbes die and decompose, a portion of these organic N compounds including amino sugars can be released as NH_4^+ through mineralization, which may reduce crop response to further applications of fertilizer N. By using a modified Illinois Soil Nitrogen Test (hydrolyzation soil with NaOH, heating to liberate NH_3, and finally analysis using acidimetric titration), Williams et al. (2007) found a highly and significantly negative correlation between soil amino sugar content to fertilizer-N response, which suggests that this form of organic N is readily mineralized during the growing season and may be used as an indicator for soil N mineralization.

The objectives of this study are to determine how cropping history (current and previous crops in a rotation) influences soil N mineralization dynamics and to examine whether soil amino sugars could be used as an indicator of soil N mineralization.

Materials and Methods

Soils

Soil samples were collected from a crop rotation experiment, which was initiated in the fall of 2001 at the Eugene F. Whelan Experimental Farm, Woodslee, Ontario (42°13′N, 82°44′W). The climate of this area is humid continental according to the Köppen climate classification (Peel et al. 2007). The mean annual temperature is 8.9 °C, and mean annual precipitation is 832 mm (Drury et al. 2009). The soil is classified as an Orthic Humic Gleysol in Canadian System of Soil Classification and a mesic Typic Argiaquoll in the USDA Soil Taxonomy. The soil texture is 280 g sand·kg^{-1}, 350 g silt·kg^{-1}, and 370 g clay·kg^{-1} in the top 20 cm.

The crop rotation experiment was arranged in a randomized complete block design with 36 treatments and four replicates. Treatments included monoculture cropping and 2 yr, 3 yr, and 4 yr crop rotations with each phase of the rotation present every year. Each plot was 20 m long and 6.1 m wide. Soil samples were taken and incubated twice separately in 2011 and 2015. Treatments selected in 2011 for the incubation included monoculture corn (C), monoculture soybean (S), monoculture winter wheat (WW), monoculture WW with underseeded red clover (*Trifolium pretense* L., RC), a 2 yr S-WW rotation, a 2 yr S-WW + RC rotation, a 3 yr C-S-WW rotation, a 3 yr C-S-WW + RC rotation, a 3 yr S-S-WW rotation, and a 3 yr S-S-WW + RC rotation. All crop phases of the rotations were included, which resulted in a total of 20 combinations of the current crop and previous crop. Red clover (14 kg seeds·ha^{-1}) was broadcast into the designated winter wheat plots on 14 April 2011. Ammonium nitrate was broadcast on the winter wheat plots on 11 May 2011 to provide 90 kg N·ha^{-1}, and wheat was harvested on 14 July 2011. Corn was planted (76 800 seeds·ha^{-1}) on 3 June 2011, and at the same time starter fertilizer was injected 5 cm beside and 5 cm below the seed to provide 20.3 kg N·ha^{-1}, 35.5 kg P·ha^{-1}, and 33.7 kg K·ha^{-1}. At the six-leaf stage, corn also received 150 kg N·ha^{-1} of liquid urea-ammonium nitrate, which was injected into the soil on 30 June 2011. Soybean was planted at 487 000 seeds·ha^{-1} on 9 June 2011 with no fertilizers. On 12 July 2011, 30 soil plugs with a diameter of 3.4 cm were randomly collected (including plant rows and space between rows) from 0 to 10 cm depth of each replicate plot. The soil plugs from the same plot were composited, placed in plastic bags in the field, and kept cool until processed in the laboratory.

Treatments selected in 2015 for the incubation included monoculture S, a 2 yr C-S rotation, a 2 yr S-WW rotation, a 2 yr S-WW + RC rotation, a 3 yr S-S-WW rotation, and a 3 yr S-S-WW + RC rotation. The samples were collected within each phase of these rotations. Red clover (14 kg seeds·ha^{-1}) was broadcast into the designated winter wheat plots on 15 April 2015, and the winter wheat plots were fertilized with ammonium nitrate at a rate of 90 kg N·ha^{-1} on the same day. Winter wheat was harvested on 22 July 2015. Corn (76 822 seed·ha^{-1}) was planted on 22 May 2015 with a 40 kg N·ha^{-1} starter and side-dressed with liquid urea-ammonium nitrate at 150 kg N·ha^{-1} on 12 June 2015. Soybeans were planted (589 159 seeds·ha^{-1}) on 27 May with no fertilizers. More detailed information on crop management and agronomy was given by Drury et al. (2008) and Agomoh et al. (2020). The second incubation trial followed the same soil sampling protocols as described above, with the soils sampled on 11 May 2015. All soils were passed through a 4 mm sieve, homogenized, and stored at 4 °C. Visible root and fresh litter material were removed from samples prior to sieving. Field-moist subsamples were used for N mineralization incubation.

Nitrogen mineralization

A long-term aerobic incubation was used to study N mineralization. Field-moist soil samples (20 g on an oven-dry basis) were weighted into 80 mL cylindrical containers (Starplex Scientific Inc., Etobicoke, ON, Canada), adjusted to a water content of 26.2% (correspond to 60% of the field capacity) with double-distilled water, and incubated at 20 °C for 28 wk in a growth chamber. The container was covered with parafilm, and five holes were made in the parafilm to maintain an aerobic environment while minimizing water loss. The soil water content was adjusted twice a week by weighing and adding water as required. Containers were destructively sampled after 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, and 28 wk, and the soils were analyzed for inorganic N (NH_4^+-N and NO_3^--N + NO_2^--N). Concentrations of NH_4^+-N and NO_3^--N + NO_2^--N in KCl (2 mol·L^{-1}) extracts were determined using the Berthelot reaction and the cadmium reduction methods, respectively, on a TrAAcs 2000 autoanalyzer (SEAL Analytical Inc., Mequon, WI, United States) (Drury et al. 2016). The 2015 incubation study was performed in the same manner, with the exception that the inorganic soil N concentrations analysis was performed on a Lachat QuickChem 8500 Flow Injection Analyzer (Hach, Loveland, CO, United States) (Yang et al. 2020). Net mineralizable N was defined as the increase in inorganic N concentration over 28 wk of incubation under constant environmental conditions. Calculation of a potentially mineralizable N pool using first-order kinetics was not possible since a significant number of the treatments followed a linear increase in inorganic N over 28 wk of incubation.

Amino sugar analysis

Amino sugar analysis was conducted based on the procedure of Zhang and Amelung (1996). Briefly, field soil samples were first hydrolyzed with 6 mol·L^{-1} HCl at 105 °C for 8 h, and then the hydrolysate was purified using filtration and then neutralized. The supernatant was freeze-dried and washed with methanol to recover the amino sugars. The amino sugars were transformed into aldononitrile derivatives, and they were separated on a Varian 450 gas chromatography (GC) equipped with an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm) and a flame ionization detector. Myo-inositol was an internal standard, which was added to the samples prior to purification and used to quantify amino sugar concentrations. A total of three amino sugars (glucosamine, galactosamine, and muramic acid) were quantitatively analyzed, and the total amino sugar content was calculated as the sum of three amino sugars. The impacts of cropping treatments on soil amino sugars were evaluated in a previous study (Zhang et al. 2014), whereas this study focused on the relationship between amino sugars and soil N mineralization. Soils collected in the second soil sampling in 2015 were not analyzed for amino sugars composition.

Statistical analysis

All statistical analyses in 2011 were performed using the R program (version 4.0.2). One-way analysis of variance (ANOVA) procedures with Tukey's HSD test as post hoc were used to test the treatment effects on soil initial N contents. Repeated measure of ANOVA was

Table 1. Soil inorganic nitrogen (N) contents (at the start of incubation) under different monoculture and rotational cropping systems in 2011.

Crop rotation	Inorganic N (mg N·kg^{-1})	Crop rotation	Inorganic N (mg N·kg^{-1})
Monoculture cropping		3 yr rotations	
Monoculture **C**	52.5 (3.4)a	3 yr **C**-S-WW rotation	49.4 (5.0)a
Monoculture **S**	10.3 (1.5)c	3 yr C-**S**-WW rotation	10.9 (0.4)c
Monoculture **WW**	12.3 (2.1)c	3 yr C-S-**WW** rotation	4.5 (0.4)d
Monoculture **WW + RC**	7.6 (0.8)cd	3 yr **C**-S-WW + RC rotation	53.4 (7.8)a
—	—	3 yr C-**S**-WW + RC rotation	13.0 (1.3)bc
2-yr rotations	—	3 yr C-S-**WW + RC** rotation	5.6 (1.1) d
2-yr **S**-WW rotation	13.1 (0.8)bc	3 yr **S**-S-WW rotation	11.4 (1.0)c
2-yr S-**WW** rotation	7.0 (0.5)cd	3 yr S-**S**-WW rotation	13.2 (1.0)bc
2-yr **S**-WW + RC rotation	23.1 (2.4)b	3 yr S-S-**WW** rotation	4.3 (0.4)d
2-yr S-**WW + RC** rotation	5.2 (0.7)d	3 yr **S**-S-WW + RC rotation	18.1 (1.4)b
—	—	3 yr S-**S**-WW + RC rotation	14.9 (1.2)bc
—	—	3 yr S-S-**WW + RC** rotation	6.6 (0.7)cd

Note: C, corn; S, soybean; WW, winter wheat; WW + RC, winter wheat underseeded into red clover. Data are means with standard errors in parentheses (n = 4). Different lowercase letters indicate statistical differences among crop rotation treatments. Underlined and bolded indicates which phase in the crop rotations.

performed to test significant differences among treatments in net N mineralization. Multiple comparisons were done with the "HSD.test" function in the "agricolae" package of the R program. Pearson correlation was conducted to examine the relationship between mineralizable N and total and individual amino sugars. In 2015, the statistical analysis was performed using SAS procedures, following the same statistical criteria as described in 2011 (Tukey, $P < 0.05$). Treatment difference in net mineralization was analyzed using the N mineralization data from week 28 of the incubation, which was the cumulative mineralization. The treatment analysis was separated by phase, with soybean plots being analyzed separately from the wheat phase. In addition, contrast and estimate statements were generated on plots comparing the presence or absence of the RC cover crop at a $P < 0.05$. The Levene's test and Shapiro–Wilk test were used to test homogeneity of variance and distribution of normality. When necessary, data were log-transformed to reduce heteroskedasticity and achieve normality.

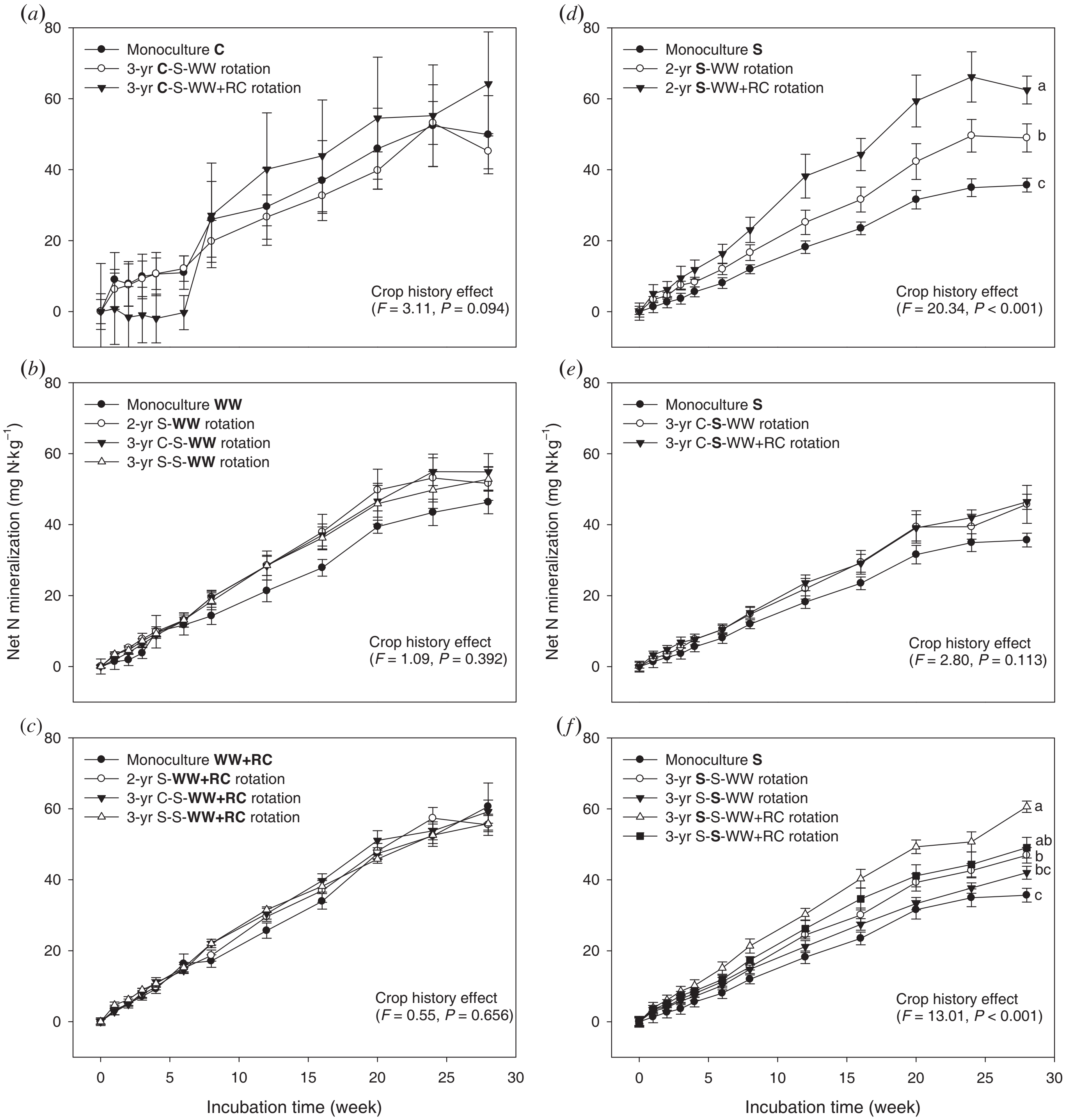
Results and Discussion

Soil inorganic N and net N mineralization – Trial 1 (2011)

The NH_4^+-N concentrations ranged between 0.5 and 2.2 mg N·kg^{-1} and changed little over the 28 wk incubation for all treatments (data not shown). This suggests that nitrification was rapid enough to prevent accumulation of NH_4^+-N. In contrast, the NO_3^--N + NO_2^--N ranged between 3.7 and 50.5 mg N·kg^{-1} and accounted for 83%–99% of the total inorganic N. The initial inorganic N contents were generally the highest (49.4–53.4 mg N·kg^{-1}) when the current crop was corn, intermediate (10.3–23.1 mg N·kg^{-1}) when the current crop was soybean, and the lowest (4.3–12.3 mg N·kg^{-1}) when the current crop was winter wheat or winter wheat underseeded into red clover (Table 1). These initial soil inorganic N results were impacted by the application dates and quantities of N fertilizers. Corn was side-dressed with N fertilizers on 30 June 2011 at 150 kg N·ha^{-1}; thus soil inorganic N levels were expected to still be high when samples were collected on 12 July 2011 just 12 days after side-dress N application. Winter wheat also received inorganic N but at a lower rate of 90 kg N·ha^{-1} on 11 May 2011 and during the two-month interval between N application and soil sampling, some of the applied N would be taken up by the winter wheat, which in turn may decrease soil inorganic N levels (Yan et al. 2020). Some of the applied N could also have been lost from soils via denitrification and (or) nitrate leaching. No N was applied to the soybean crop, but soil inorganic N was still higher in the soybean phases than the wheat phases. This can be explained by the release of N, which was symbiotically fixed by the leguminous soybean crop (Rodriguez et al. 2020).

We did not find a significant effect of the initial inorganic N concentration on soil N mineralization; thus the initial inorganic N contents were scaled to zero to compare net N mineralization among treatments (Fig. 1). The effect of cropping history on soil net N mineralization was found to be dependent on both the current and previous crops. Net N mineralization over the 28 wk was generally similar when the current crop was corn (F = 3.11, P = 0.094), even though it did not change over the first 5 wk when the previous crop was winter wheat + red clover (Fig. 1*a*). This suggests that over the entire incubation period, soil N mineralization was not influenced by cropping history in the corn phase of the rotation. The net N mineralization was not significantly different among treatments when the current crop was winter wheat (F = 1.09, P = 0.392, Fig. 1*b*) or winter wheat

Fig. 1. Net change in soil inorganic nitrogen (N) under different crop rotations in 2011. Error bars indicate standard errors. The lowercase letters indicate significant differences among treatments. The figures were separated based on current crops.



underseeded with red clover ($F = 0.55$, $P = 0.656$, Fig. 1*c*). This suggests soil N mineralization was not influenced by cropping history when WW or WW + RC was the current phase of the rotation, although the net N mineralization was greater when the current crop was WW + RC (55.5–60.6 mg N·kg^{-1}) compared with WW (46.3–54.9 mg N·kg^{-1}). This is consistent with the results of Gentry et al. (2013), who found a considerable N credit from red clover at 30–48 kg N·ha^{-1} in two separate years, suggesting the benefit of legume crop in supplying additional N. In the soybean phase of the 2 yr rotations, the S-WW (49.0 mg N·kg^{-1}) and S-WW + RC (62.5 mg N·kg^{-1}) rotations resulted in significantly greater mineralization rates than monoculture soybean

(35.7 mg N·kg^{-1}), especially when the previous crop was WW + RC (F = 20.34, P < 0.001, Fig. 1*d*). Net mineralization was higher but not significantly different in soils under the 3 yr C-S-WW and the 3 yr C-S-WW + RC rotations compared with monoculture soybean (F = 2.80, P = 0.113, Fig. 1*e*). The 3 yr S-S-WW (first year S) and 3 yr S-S-WW + RC rotations (both first and second year) also increased the net N mineralization compared with monoculture soybean (F = 13.01, P < 0.001, Fig. 1*f*). In particular, the first-year soybean phase of the S-S-WW + RC rotation had significantly greater net N mineralization than all other rotations except the corresponding second year soybean phase for the S-S-WW + RC rotation (Fig. 1*f*). Monoculture soybean was lower than all other 3 yr rotations that had 2 yr of S except for the second-year phase of the S-S-WW rotation (Fig. 1*f*). These results indicate that (*i*) planting WW before S increased the N supplying capacity of the soil, (*ii*) interseeding RC to WW before soybean further increased the N supplying capacity of soil significantly during the soybean growing season, and (*iii*) planting corn before soybean resulted in similar soil N mineralization rates as compared with monoculture soybean. In addition to the S-S-WW rotations (with or without RC), the first year of soybean had a greater net N mineralization rate than the second year of soybean, which probably indicates a reduced legacy effect of WW or WW + RC.

Soil inorganic N and net N mineralization – Trial 2 (2015)

Similar to the 2011 results, the NH_4^+-N contribution to inorganic N was negligible compared with the NO_3^--N in the soil. At time 0, NH_4^+-N ranged from 0.1 to 2.2 mg N·kg^{-1}, accounting for 0.3%–16% of initial soil inorganic N. The NH_4^+-N levels decreased gradually through nitrification and ranged from 0 to 1.5 mg N·kg^{-1} at the end of the incubation. There was no significant effect of crop rotation or phase within rotation on these low NH_4^+-N concentrations (data not shown).

Since NH_4^+-N constituted a small portion of the total inorganic N, it was combined with NO_3^--N data and presented as total inorganic N. The treatment differences (significance and magnitude) found in the NO_3^--N data (data not shown) were the same as the total inorganic *N* values. Total inorganic *N* values at time 0 were higher in the WW and WW + RC treatments (48.7–61.1 mg N·kg^{-1}) when compared with the soybean and corn phases (10.5–23.3 mg N·kg^{-1}) (Tables 2 and 3). This was expected as the winter wheat plots received 90 kg N·ha^{-1} on 15 April 2015, which still had elevated soil inorganic N conditions when the soils were sampled on 11 May 2015. This suggests the effect of fertilizer on soil inorganic N concentrations can last for almost one month, but not exceed two months, considering the situation in 2011 soils. There were no significant differences in initial inorganic N between the WW and WW + RC plots, which was in agreement with the 2011 samples. This suggests that while the RC was actively growing (planted on 15 April), it had not increased soil N at that sampling time. At the end of the incubation, there were no significant differences between the WW and WW + RC for total inorganic N (Table 3). Total inorganic N was significantly impacted by treatments at time 0 in the soybean phases of the rotations (Table 2). The initial

Table 2. Initial and final soil inorganic nitrogen (N) values over a 28 wk incubation on soils within the soybean phase of the Totten Rotation sampled in 2015.

	Soil inorganic N (mg N·kg^{-1})	
Crop rotation	Initial	Final
Monoculture **S**	16.3 (0.7)abc	62.1 (3.0)cd
2 yr C-**S** rotation	11.0 (0.6)c	60.9 (1.4)d
2 yr **S**-WW rotation	14.8 (2.2)bc	64.3 (4.6)bcd
2 yr **S**-WW + RC rotation	23.3 (2.0)a	85.2 (4.4)a
3 yr **S**-S-WW rotation	12.3 (0.9)c	64.4 (2.6)bcd
3 yr S-**S**-WW rotation	15.0 (1.3)abc	74.0 (1.0)abcd
3 yr **S**-S-WW + RC rotation	18.5 (2.2)abc	76.0 (4.1)abc
3 yr S-**S**-WW + RC rotation	22.4 (2.9)ab	81.2 (5.7)ab
Pr > F	<0.001	<0.001
Contrast RC vs no RC (Monoculture soybean and 2 yr C-S rotation excluded)		
Contrast	7.4	14.4
Estimate	< 0.001	< 0.001

Note: C, corn; S, soybean; WW, winter wheat; WW+ RC, winter wheat underseeded into red clover. Numbers in brackets are standard error (n = 4). Different lowercase letters indicate statistical differences among treatments in a column. Underlined and bolded indicate which phase in the 3 yr rotations.

Table 3. Initial and final soil inorganic nitrogen (N) values over a 28 wk incubation on soils within the winter wheat and corn phase of the Totten Rotation in 2015.

	Soil inorganic N (mg N·kg^{-1})	
Crop rotation	Initial	Final
2 yr S-**WW** rotation	51.8 (6.8)a	107.9 (9.2)a
2 yr S-**WW + RC** rotation	61.1 (1.9)a	115.3 (0.5)a
3 yr S-S-**WW** rotation	58.4 (3.6)a	97.9 (1.8)a
3 yr S-S-**WW + RC** rotation	48.7 (1.2)a	108.2 (3.1)a
2 yr **C**-S rotation	10.5 (1.3)	60.0 (5.7)
Pr > F	NS	NS
Contrast RC vs no RC (2 yr C-S rotation excluded)		
Contrast	NS	NS
Estimate	NS	NS

Note: C, corn; S, soybean; WW, winter wheat; WW + RC, winter wheat underseeded into red clover. Numbers in brackets are standard error (n = 4). Different lowercase letters indicate statistical differences among treatments in a column (2 yr C-S rotation excluded). Underlined and bolded indicate which phase in the 3 yr rotations.

soil inorganic N was significantly greater in the 2 yr S-WW + RC rotation (23.3 mg N·kg^{-1}) than in the 2 yr C-S rotation, the 2 yr S-WW rotation, and the first-year soybean phase of the 3 yr S-S-WW rotation (11.0–14.8 mg N·kg^{-1}). The lowest initial soil inorganic N was found in the 2 yr C-S rotation at just 10.5 mg N·kg^{-1} for the corn and 11.0 mg N·kg^{-1} for the soybean phases of the rotation (Tables 2 and 3). The contrast comparison between S-WW, S-S-WW and S-WW + RC, S-S-WW + RC was significant at time 0 for total inorganic N, with the presence of RC increasing N by 52% (Table 2). This contrast included plots with RC being terminated in the fall of 2014 (2 yr S-WW + RC and first-year soybean phase of the 3 yr S-S-WW + RC) and plots with RC being terminated in the fall of 2013 (second-year soybean phase of the 3 yr S-S-WW + RC). This suggests that it was no longer the direct effect of the previous cover crop supplying the N in June, but rather the mineralization of soil organic matter provided at least in part some of this inorganic N. After 28 wk, the 2 yr S-WW + RC had sustained significantly greater N (85.2 mg N·kg^{-1}), compared with monoculture S, 2 yr C-S, 2 yr S-WW, and the first-year soybean in the 3 yr S-S-WW (60.9–64.4 mg N·kg^{-1}). This can be largely attributed to the decomposition of red clover, which consequently contributed to the N release into the soil. A recent study reported an average inorganic N of 7 kg·ha^{-1} was released from red clover with in situ labeling incubation (Notaris et al. 2020). Similarly, the presence of RC in rotation increased total inorganic N by 20% over the wheat rotations without RC in our study at the end of incubation (Table 2).

Net N mineralization within the soybean phase was the highest in the 2 yr S-WW + RC plots at 61.9 mg N·kg^{-1}, being only significantly greater than the monoculture S plots at 45.8 mg N·kg^{-1} (Fig. 2*a*). This significant difference was similar to the finding found in 2011 (Fig. 1*d*); however, in this case the net N mineralization in the 2 yr S-WW + RC and 2 yr S-WW rotations did not differentiate despite it being 25% greater in the 2 yr S-WW + RC than the 2 yr S-WW. The lower mineralization rates from the monoculture S are likely due to the lower soil organic matter (Zhang et al. 2014) occurring in these plots. This will largely be due to small residue return with soybean compared with wheat, corn, and RC, indicating that monoculture S did not contribute to the soil organic N pool. This is consistent with the finding that monoculture soybean had a significantly lower soil organic C and N compared with rotations with sorghum and corn over a 10–12 yr study (Havlin et al. 1990). Using the contrast statement, there was a 13% increase in net N mineralization when RC was present in rotation (S-WW + RC, S-S-WW + RC vs. S-WW, S-S-WW) (P = 0.016). Using a typical bulk density for this

Fig. 2. Net nitrogen (N) mineralization of the soybean phase in the 2 yr rotations (*a*), net N mineralization of the soybean phase in the 3 yr rotations (*b*), and net N mineralization of soils when the current crop was WW, WW + RC, or corn (*c*) within the Totton Rotation over a 28 d period on soil samples in 2015. Treatments in (*a*) and (*b*) were analyzed together and presented separately for clarity. Error bars indicate standard errors. The lowercase letters indicate significant differences among treatments. S, soybean; C-S, 2-yr corn-soybean rotation; S-WW, 2-yr soybean-winter wheat rotation; S-WW + RC, 2-yr soybean-winter wheat + red clover rotation.

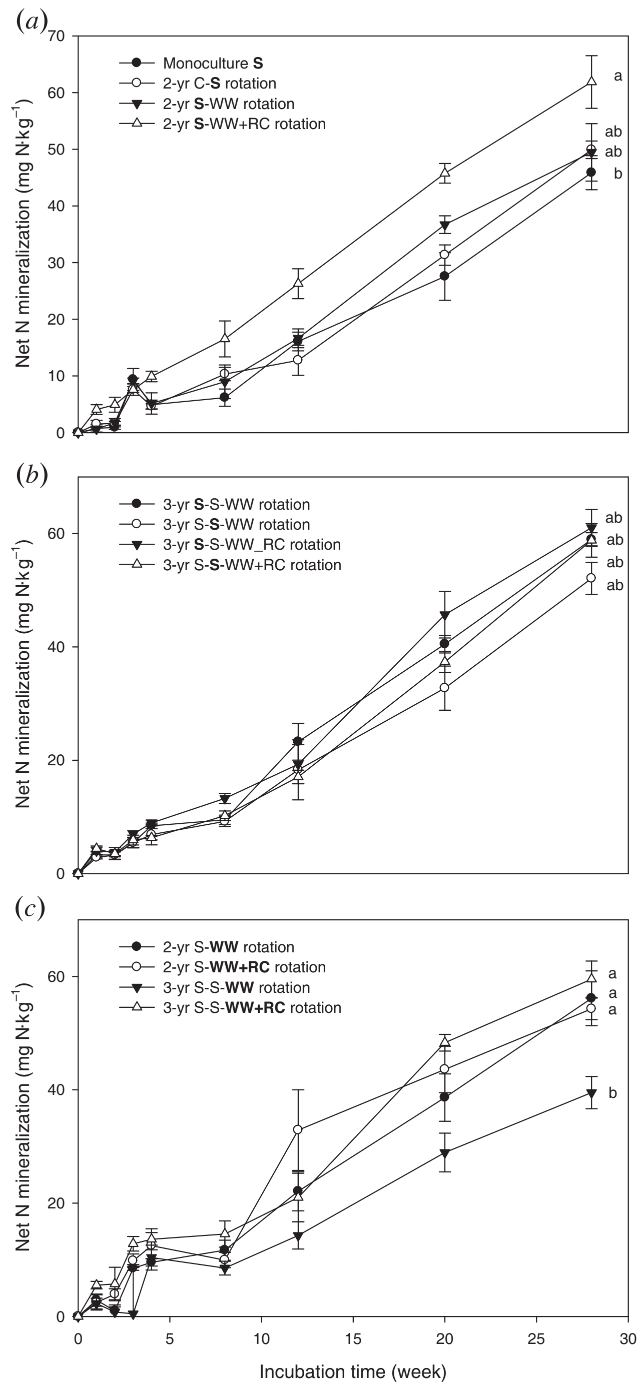
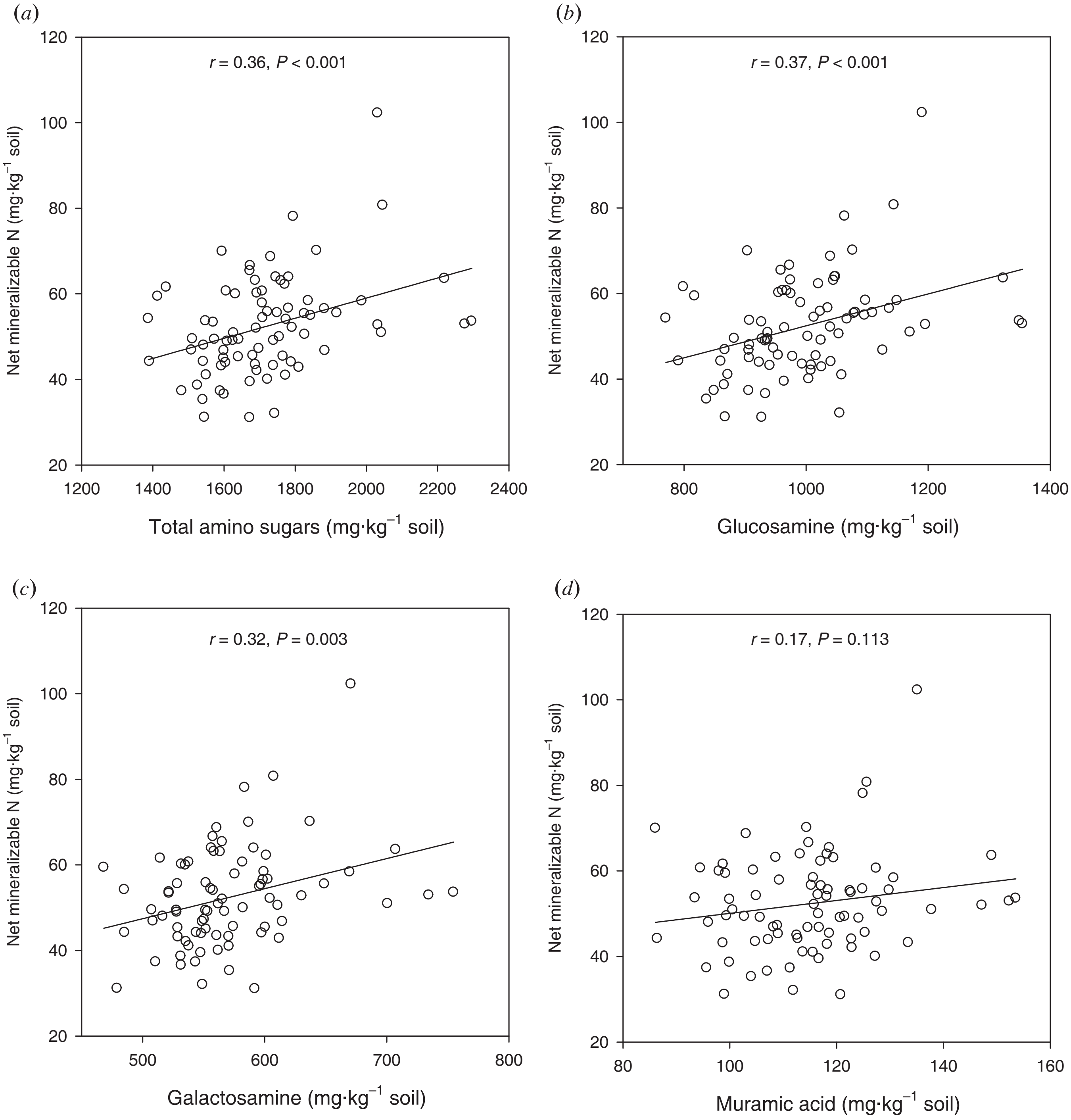


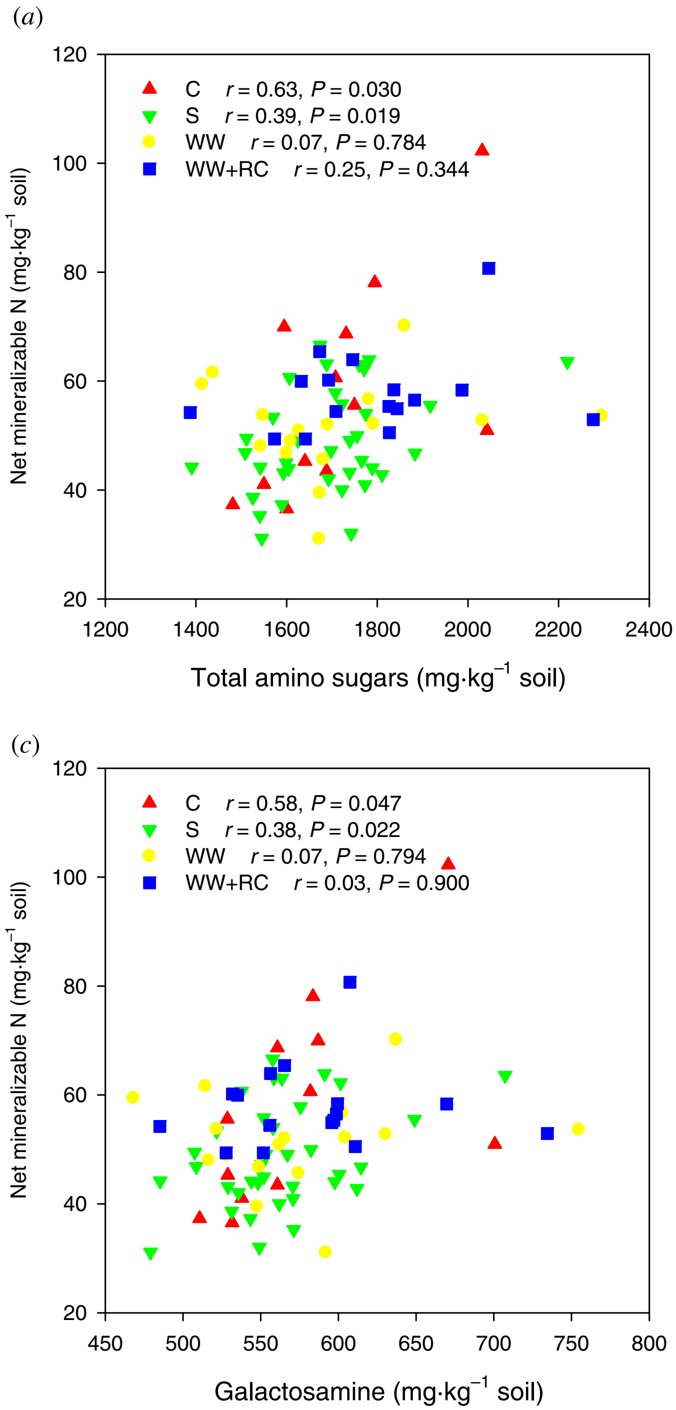
Fig. 3. Correlations between net mineralizable nitrogen (N) and amino sugars with all data from samples collected in 2011.



field site of 1.36 $Mg \cdot m^{-3}$ (Drury et al. 2003), plots with RC were supplying an estimated 9.6 kg $N \cdot ha^{-1}$ more N as compared with plots without RC at the 0–10 cm depth. The mineralization patterns exhibited a near-linear release pattern as of 28 wk (Fig. 2), suggesting that the N supply can be sustained for a longer period before a plateau occurs and as in 2011, hence a kinetic curve could not be calculated.

In the winter wheat phase of the rotations, the net N mineralization of 3 yr S-S-WW was significantly lower than all other wheat treatments (Fig. 2*c*). The low net N mineralization of the 3 yr S-S-WW rotation was potentially due to the frequency of the low biomass return of soybean, as well as the absence of an ameliorating effect of the RC to supplement total biomass and N into the system. This was supported by a recent

Fig. 4. Correlations between net mineralizable nitrogen (N) and amino sugars based on data when the current crop was corn (C), soybean (S), winter wheat (WW), and winter wheat + red clover (WW + RC) from samples collected in 2011. [Colour online.]



study of soil health indicators from this same experiment in which the soybean rotations tended to have lower potentially mineralizable N when comparing to continuous winter wheat (Agomoh et al. 2020). The presence of RC in rotation was a significant contrast with a 19% increase in net N mineralization, with an increased potential supplying capacity of 12.3 kg $N \cdot ha^{-1}$ in the 0–10 cm depth over a 28 wk period (P = 0.010, Table 3). The net N mineralization was significantly higher in both phases of the 2 yr S-WW + RC rotation in 2015 but only in the soybean phase of that in 2011. Given that the contrasts showed that RC always increased N mineralization, this result could be an indication that in those 4 yr between samplings, these changes due to RC were becoming a rotational effect from soil organic matter cycling rather than just being affected by the crop residue decomposition in that year.

Relationship between net mineralizable N and amino sugars

The amino sugar data for individual rotation treatments was published in our previous study and thus not reported here (Zhang et al. 2014). However, when the entire data set was evaluated, significant correlations were found between net mineralizable N and total amino sugars (r = 0.36, P < 0.001, Fig. 3*a*), glucosamine (r = 0.37, P < 0.001, Fig. 3*b*), and galactosamine (r = 0.32, P = 0.003, Fig. 3*c*), but not muramic acid (r = 0.17, P = 0.113, Fig. 3*d*). The significant correlation of N mineralization with total amino sugars was primarily due to its correlation with glucosamine and galactosamine because more than 90% of the total amino sugars were composed of these two amino sugars (Zhang et al. 2014). Our result was consistent with the studies that found significant linear relationships between hydrolyzable amino sugar-N and soil N mineralization using both the Illinois Soil N Test and the Direct Steam Distillation procedure (Bushong et al. 2008; Roberts et al. 2009). The lower correlation coefficients in these studies were probably because other organic N fractions, such as amino acids, contributed to soil N mineralization (Zhang et al. 2015). A correlation was conducted based on the current crop phase to relate net mineralizable N to amino sugar concentrations. We found significant relationships between net mineralizable N and total amino sugars (P = 0.030), glucosamine (P = 0.032), and galactosamine (P = 0.047) when the current crop was corn (Fig. 4). Net mineralizable N was also significantly correlated with total amino sugars (P = 0.019), glucosamine (P = 0.012), and galactosamine (P = 0.022) when the current crop was soybean (Fig. 4). However, net mineralizable N was not correlated with total amino sugars, glucosamine, galactosamine, or muramic acid when the current crop was winter wheat with/without red clover (Fig. 4, P > 0.05). This was particularly interesting as total amino sugars were greater in soils under monoculture WW and WW + RC as compared with monoculture corn and soybean (Zhang et al. 2014). One possible explanation was the differences in tillage intensity, which may protect amino sugars from mineralization. Winter wheat plots were only tilled in the fall, whereas corn and soybean plots had both fall primary and spring secondary tillage; therefore amino sugars were more protected by soil aggregates in WW plots, and the protection by aggregates may have reduced mineralization during the incubation. In contrast, the intensive tillage in corn and soybean plots may have led to greater exposure of amino sugars to microbial attack when nutrients were exhausted during incubation, thus resulting in better correlation between amino sugars and N mineralization. Our results suggest that amino sugars, especially total amino sugar content, could be a good indicator for net N mineralization in tilled soils when the current crop was corn and soybean.

Conclusions

Crop types influence the nutrients and carbon return to the soil and consequently exert strong effects on soil N supplying capacity. Our study indicates that N supplying capacity of soil was influenced by both current and previous crops in a rotation. Specifically, net soil N mineralization was generally similar when the current crop was corn, irrespective of the previous crop, even if it was a legume crop. In addition, when corn was the previous crop, net soil N mineralization was not affected. However, planting WW before soybean, especially when underseeded with RC, significantly increased net soil N mineralization for both the 2 yr and 3 yr rotations; this was evident in both 2011 and 2015 samplings. We also identified a significant correlation between amino sugar content and mineralizable N, especially in the corn and soybean phases of crop rotations, which suggests that total amino sugar content could serve as an indicator for N supplying capacity in tilled soils. Further, since amino sugar analysis does not require a 28 wk incubation like the mineralization assay, it would provide a more timely method to predict N supply in varying cropping systems.

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Conflict of Interest

The authors declare there are no conflicts of interest.

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