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Soil nitrogen fractions under long-term crop rotations in the Loess Plateau of China

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ABSTRACT

Long-term crop rotation may influence soil N storage, mineralization, and availability. We studied the 30 yr effect of crop rotations on soil N fractions at 0–15 and 15–30 cm depths in the Loess Plateau of China. Crop rotations were continuous winter wheat (*Triticum aestivum* L.) (W), corn (*Zea mays* L.)-winter wheat-winter wheat-millet (*Eleusine coracana* L.) (CWWM), pea (*Pisum sativum* L.)-winter wheat-winter wheat-millet (PWWM), and sainfoin (*Onobrychis viciifolia* Scop.)-winter wheat-winter wheat-sainfoin (SWWS); pea-winter wheat-winter wheat-corn (PWWC); alfalfa (*Medicago sativa* L.) (4 yr)-potato (*solanum tuberosum* L.) (1 yr)-winter wheat (3 yr) (A4PoW3), and fallow (F). Nitrogen fractions were soil total N (STN), particulate organic N (PON), microbial biomass N (MBN), potential N mineralization (PNM), NH_4–N, and NO_3–N. The STN and PON at 0–15 cm were greater in CWWM and at 15–30 cm were greater in A4PoW3 than F and W. The PNM at both depths was greater in A4PoW3 than other crop rotations, except SWWS and CWWM. The MBN was greater in CWWM, PWWM, SWWS, and A4PoW3 than other crop rotations. The NH_4-N content was greater in F than other crop rotations, except PWWC. The NO_3-N content at 0–15 cm was greater in CWWM and at 15–30 cm was greater in PWWM than F. Most soil N fractions were correlated with each other and also with the length of the crop rotation. Diversified crop rotations with increased root biomass N returned to the soil and longer year rotations enhanced soil N storage, mineralization, and availability compared with monocropping and fallow.

1. Introduction

Nitrogen is a major limiting nutrient for achieving sustainable crop yields in dryland cropping systems (Janzen et al., 2003; Lenssen et al., 2007; Sainju et al., 2009). Nitrogen fertilization can enhance crop yields and quality, but excessive fertilization can reduce soil and environmental quality by increasing soil acidification, N leaching, and greenhouse gas (N_2O) emissions (Ross et al., 2008; Sainju et al., 2016). As crops can remove about 40 to 60% of applied N from inorganic N fertilizer, manure and other N amendment, the residual soil N (NO_3-N + NH_4-N) after crop harvest can be lost to the environment (Janzen et al., 2003; Ross et al., 2008). Because soil residual and potentially mineralizable N can also contribute N to crops during the growing season, it is necessary to adjust N fertilization rates so that crop production can be optimized and potential for N losses minimized (Sarker et al., 2015; Akhter et al., 2016; Sainju et al., 2016). While some of the residual N is immobilized by microorganisms into soil organic N, unharvested N in crop residue (stems and leaves) and roots recycle to form the core of soil N storage. By increasing N-use efficiency, enhancing N storage, and reducing N fertilization rate through improved management practices, N losses to the environment can be minimized compared with traditional practices (Janzen et al., 2003; Ross et al., 2008; Sainju et al., 2012, 2016). By understanding soil N cycling, N fertilization to crops can be managed efficiently for sustaining crop yields and quality and reducing the potential for N loss (Sarker et al., 2015; Akhter et al., 2016; Sainju et al., 2016).

Crop rotation can enhance crop yield and quality by efficiently utilizing water and N compared with monocropping in water-limited dryland farming systems (Sainju et al., 2012). Legumes, such as pea, in rotation can not only reduce N fertilization rates to successive crops by

Abbreviations: A4PoW3, alfalfa (4 yr)-potato (1 yr)-winter wheat (3 yr); CWWM, corn-winter wheat-winter wheat-millet; F, fallow; MBN, microbial biomass N; PNM, potential N mineralization; PON, particulate organic N; PWWC, pea-winter wheat-winter wheat-corn; PWWM, pea-winter wheat-winter wheat-millet; STN, soil total N; SWWS, sainfoin-winter wheat-winter wheat-sainfoin; W, continuous winter wheat

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supplying N from their residues due to higher N concentrations, but also utilize soil water more efficiently than nonlegumes, such as wheat and barley (*Hordeum vaularis* L.) (Sainju et al., 2012; Lenssen et al., 2007, 2014; Hossain et al., 2016; Jahan et al., 2016). Deep-rooted perennial legume forages, such as alfalfa, can also scavenge residual soil N and reduce N leaching (Karlen et al., 1994; Sainju and Lenssen, 2011). After termination of such crops, more N becomes available to successive crops from residue decomposition due to higher N concentrations in legumes than nonlegumes (Hossain et al., 2016; Jahan et al., 2016). Some rotations, however, may temporarily reduce plant available N by increasing N immobilization when crops with higher C/N ratio are included in the rotation (Zhang et al., 2007). Perennial legume forages can also enrich soil N storage by increasing N inputs from root biomass because of their extensive root systems compared with annual crops (Summers, 1998; Zentner et al., 2011).

Because of a large pool size and inherent spatial variability, changes in soil total N (STN) due to management practices are often slow (Franzluebbers et al., 1995; Sainju et al., 2011). As a result, changes in STN do not adequately reflect soil productivity and N status (Franzluebbers et al., 1995; Sainju et al., 2009; 2011). The microbial biomass N (MBN), as a measure of N immobilization, and potential N mineralization (PNM), as a measure of N mineralization, are labile soil N fractions that change rapidly during a crop growing season (Sainju et al., 2009). The particulate organic N (PON), a measure of N storage in coarse organic matter, is an intermediate fraction between slow and labile fractions that also changes rapidly during a growing season and is an important substrate for soil microorganisms (Zhang et al., 2007). The soil mineral N (NO_3-N and NH_4-N) is the available N fraction that can either be taken up by the crop or loss to the environment (Wood et al., 1990; Sainju et al., 2012, 2016). Increased soil residual N (NO_3-N and NH_4-N) accumulation after crop harvest can reduce water and air quality by increasing the potential for N leaching in the groundwater and increasing greenhouse gas (N_2O) emissions (Janzen et al., 2003; Ross et al., 2008; Sainju et al., 2012, 2016).

Continuous monocropping under conventional tillage with high N fertilization rates for the last several decades has resulted in soil fertility loss and erosion in the Loess Plateau of China (Huang et al., 2003; Jiang et al., 2015). In this region, winter wheat monocropping under rainfed condition accounts for 70–80% of the total crop production, however, often has low yields (Huang et al., 2003; Jiang et al., 2015). Crop rotations that can efficiently utilize soil water and N, reduce N fertilization rates, and sustain crop yields are urgently needed. Diversified crop rotations that include legumes and forages with winter wheat have been initiated in the last few decades to enhance soil fertility and crop yields (Guo et al., 2008; Cai and Hao, 2015; Jiang et al., 2015). The effect of such rotations on soil N storage, mineralization, and availability as well as N balance compared with monocropping and fallow, however, is lacking in this region.

We evaluated the long-term (30 yr) effect of diversified crop rotations that included cereals, legumes, tuber crops, and forages on soil N fractions and N balance compared with monocropping and fallow in the Loess Plateau of China. Our objectives were to: (1) quantify the impact of crop rotations of various rotation lengths on STN, PON, MBN, PNM, NH_4-N, and NO_3-N at 0–15 and 15–30 cm depths, (2) examine relationships between root biomass N input, crop rotation length, and soil N fractions, (3) evaluate N balance based on N inputs, outputs, and soil retention, and (4) determine crop rotations that enhance soil N storage, mineralization, and availability and reduce N balance compared with monocropping and fallow. We hypothesized that crop rotations with high root biomass N returned to the soil and greater rotation lengths would increase soil N fractions and reduce N balance compared with monocropping and fallow.

2. Materials and methods

2.1. Experimental site

The long-term crop rotation experiment began in September 1984 at the Changwu Agroecological Station (107°44′E, 35°12′N; 1220 m elevation) in Changwu County, Shaanxi Province, China (Guo et al., 2008; Cai and Hao, 2015; Fu et al., 2017). The experimental site has a continental monsoon climate, with mean annual temperature of 9.1°Cand the frost-free period of 194 d. The long-term (1984–2008) average annual precipitation is 580 mm, half of which occurs during July to September. The soil is a Heilutu silt loam (Calcarid Regosol according to the FAO classification system or Ultisol according to the U.S. soil taxonomy), with 45 g kg^{-1} sand, 656 g kg^{-1} silt, 309 g kg^{-1} clay, 8.4 pH, 105 g kg^{-1} $CaCO_3$, 10.5 g kg^{-1} organic C, 1.0 g kg^{-1} total N, and 1.4 Mg m^{-3} bulk density at the 0–30 cm depth at the beginning of the experiment.

2.2. Treatments and crop management

The experiment design has been described in detail by Fu et al. (2017). In brief, seven crop rotations with rotation lengths of 1–8 yr (Table 1) were arranged in a randomized complete block design with three replications. Each phase of the crop rotation was present in every year. As a result, the number of plots in each crop rotation was also proportional to the length of the crop rotation. Each plot had 10.3 m by 6.5 m size separated by 0.5 m strip, and each block was separated by 1 m strip.

Crops were planted by hand under conventional tillage using animal-drawn (first 16 yr) and hand tractor-drawn (second 14 yr) plows to a depth of 10 cm. Crops were planted at 20 cm row spacing, except for corn and potato which were planted at 70 cm spacing. Plant populations were 2.23, 0.60, 0.04, and 0.50 million plants ha^{-1} for winter wheat, pea, corn, and potato, respectively, and seeding rates were 28, 9, and 38 kg ha^{-1} for millet, alfalfa, and sainfoin respectively. At planting, chemical fertilizers were broadcasted to winter wheat, corn, millet, and potato using urea (46% N) and monoammonium phosphate (11% N, 23% P) at rates of 120 kg N ha^{-1} and 20 kg P ha^{-1}. Pea, sainfoin, and alfalfa also received N and P from monoammonium phosphate at 10 kg N ha^{-1} and 20 kg P ha^{-1}, respectively. Because of high soil K content, no K fertilizer was applied. Weed management was done by hand before, during, and after crop growth. Pesticides were applied as needed to control pests.

Winter wheat, pea, corn, and millet were harvested by hand from the entire plot by cutting plants at a height of 2 cm above the ground. A portion was oven dried at 70 °C for 7 d to determine the dry matter weight which was used as a conversion factor for calculating total biomass (grain + leaves + stems) yield. Grain yield was determined after separating grains from straw or stubble by threshing plants and oven drying a portion of the grain at 70 °C for 7 d. Biomass (stems + leaves) yield was determined by deducting grain yield from total biomass yield. Potato yield was determined by hand harvesting potato tuber from the entire plot at field moisture content after separating the aboveground biomass, a portion of which was oven-dried at 70 °C for 3 d to determine dry weight. After grain harvest, aboveground biomass (stems and leaves) of all crops were removed. Sainfoin and alfalfa were harvested by hand one to two times a year for forage from the entire plot, depending on precipitation and biomass growth. A portion was oven-dried at 70 °C for 3 d to determine dry weight, from which biomass yield was determined. Total biomass yield was determined as the sum of individual cuttings. Annualized biomass and grain yields for a rotation were calculated by dividing total biomass and grain yields of all crops by the number of crops within the rotation in a year and mean annualized biomass and grain yields were calculated by averaging annualized biomass and grain yields of all crops for a rotation across years. For grain yields in SWWS and A4PoW3, only grain yield of winter

Table 1
Description of crop rotations and planting and harvest dates of crops used in the study.

Abbreviation	Crop rotation	Length of the crop rotation (yr)	Number of plots	Planting and harvest sequence
F	Fallow	0	1	No crops
W	Continuous winter wheat	1	1	Wheat was planted in late September and harvested in the late June in each year.
CWWM	Corn-winter wheat-winter wheat-millet	3	3	Corn was planted in middle April and harvested in middle September. Winter wheat was planted in late September and harvested in early June of the second year. Winter wheat was planted in September and harvested in early June of the third year. Millet was planted in late June and harvested in middle September.
PWWM	Pea-winter wheat-winter wheat-millet	3	3	Pea was planted in middle March and harvested in early July. Winter wheat was planted in late September and harvested in early June of the second year. Winter wheat was planted in September and harvested in early June of the third year. Millet was planted in late June and harvested in middle September.
SWWS	Sainfoin-winter wheat-winter wheat-sainfoin	3	3	Sainfoin was planted in early July and aboveground biomass harvested in early September. Winter wheat was planted in late September and harvested in early June of the second year. Winter wheat was planted in September and harvested in early June of the third year. Sainfoin was planted in early July and harvested in early September.
PWWC	Pea-winter wheat-winter wheat-corn	4	4	Pea was planted in middle March and harvested in early July. Winter wheat was planted in late September and harvested in late June of the second year. Winter wheat was planted in September and harvested in late June of the third year. Corn was planted in middle April of the fourth year and harvested in middle September.
A4PoW3	Alfalfa-potato-winter wheat	8	8	Alfalfa was planted in March and terminated in February for first four years. Alfalfa biomass was harvested two to three times every year depending on growth. In the fifth year, potato was planted in early March and harvest in middle September. Winter wheat was planted in late September and harvested in late June for the next three years.

wheat was taken into account.

Because aboveground biomass of all crops was removed at harvest, belowground (root) biomass residue N and N added through N fertilizers and biological N fixation from legumes were main N inputs to the soil. As the experiment was initiated 30 yr ago and various people managed it, root biomass yield and N concentrations in above- and belowground biomass were not measured. Root biomass N and belowground biological N fixation were estimated based on root/shoot ratio and N concentrations in above- and belowground plant components for various crops available in the literature (Table 2) and aboveground biomass and grain yields (Table 3). The root biomass yield for each crop was estimated by multiplying total aboveground biomass by the root/shoot (stems + leaves + grains) ratio for each crop shown in Table 2. Root biomass N content (kg N ha^{-1}) was calculated by multiplying root biomass yield by N concentration. Mean annualized root biomass N for a crop rotation was calculated by averaging root biomass N of each crop within a rotation across years. Belowground biological N fixation for legumes (pea, sainfoin, and alfalfa) was calculated by multiplying their root biomass N by 0.70, assuming that 70% of N is biologically fixed by legumes and 30% is taken up from the soil (Sainju, 2017).

2.3. Collection and analysis of soil samples

Soil samples were collected from a depth of 30 cm from five places in central rows of each plot using a hand probe (5 cm inside diameter) in September 2014 (Fu et al., 2017). Samples were divided into 0–15 and 15–30 cm segments representing each depth, composited by depth, air-dried, sieved, and ground to 2 mm. Crop residues, root materials, and stones were removed during sieving. A separate undisturbed soil core (5 cm inside diameter) was collected at 0–15 and 15–30 cm depths from each plot at the same time. The core was oven dried at 110 °C for 24 h and weighed, from which the bulk density was calculated by dividing the weight of the oven-dried soil by the volume of the core.

The STN concentration in soil samples was determined by the combustion method using a high induction furnace C and N analyzer (Euro Vector EA3000, Manzoni, Italy) after grinding the samples to < 0.10 mm. For PON determination, 10 g soil was dispersed with 30 ml of 5 g L^{-1} sodium hexametaphosphate for 16 h (Cambardella and Elliott, 1992). The dispersed solution was poured through a 0.053 mm sieve and the retained material was rinsed with deionized water several times until all fine particles passed through the sieve. The solution and particles that passed through the sieve and contained mineral associated and water soluble N were dried at 50°C for 3–4 d and total N concentration was determined by using the analyzer as above. The PON concentration was calculated as the difference between total N concentration in whole-soil and that in the particles that passed through the sieve after correcting for the sand content. After removing coarse organic particles with a tweezer by hand, sand content was determined by oven drying sand particles retained in the 0.053 mm sieve at 110 °C for 24 h and weighing. The sand content-corrected PON concentration in the solution and particle that passed through the sieve was determined by multiplying PON concentration by sand content.

The PNM concentration was determined by the incubation method modified by Haney et al. (2004), who concluded that PNM values obtained after 10 d of soil incubation provide a good measure of N mineralization in the field as those obtained with longer incubation. In this study, two 10 g soil samples were moistened with water to 50% field capacity and incubated in a 1 L jar at 21 °C for 10 d. After 10 d, one container was removed and extracted with 50 ml of 2 mol L^{-1} KCl for 1 h. The NH_4–N and NO_3–N concentrations in the extract were determined using the modified Griess–Illosvay method with an autoanalyzer (Lachat Instruments, Loveland, CO). The PNM concentration was determined by the difference between the sum of NH_4–N and NO_3–N concentrations before and after incubation. The other container with moist soil was subsequently used for determining MBN

Table 2
Estimated root/shoot (stems + leaves + grains) ratio and N concentrations in aboveground biomass (stems + leaves), grain, and root of various crops used in the rotation available in the literature.

Crop	Root/shoot ratio in crops		N concentration in plant components			
	Root (0-120 cm)/ shoot ratio	Reference	Biomass (stems + leaves) (g N kg^{-1})	Grain (g N kg^{-1})	Root (g N kg^{-1})	Reference
Wheat	0.33	Campbell and de Jong (2001)	16.5	18.0	10.0	Katterer et al. (1993)
Corn	0.35	Bollinder et al. (1997)	12.5	15.5	8.3	Crozier and King (1993)
Millet	0.26	Sainju and Lenssen (2011)	19.0	23.0	11.7	Sainju and Lenssen (2011)
Pea	0.17	Jacob et al. (2017)	20.2	35.0	12.5	Sainju et al. (2009)
Sainfoin	0.65	Bollinder et al. (2002)	24.0	–	13.1	Baltensperger and Smith, (1984)
Alfalfa	0.95	Bollinder et al. (2002)	25.0	–	14.8	Sainju and Lenssen (2011)
Potato	0.18	Wishart et al. (2013)	16.0	–	10.0	Katterer et al. (1993)

Table 3
Mean annualized crop biomass (stems and leaves) and grain yields across years, estimated annualized belowground (root) biomass N returned to the soil, and total N input as affected by crop rotation.

Crop rotation[a]	Mean annualized biomass yield (Mg ha^{-1})	Mean annualized grain yield (Mg ha^{-1})	Estimated mean annualized root biomass N (0-120 cm) returned to the soil (kg N ha^{-1})	Total N input[b]
W	7.9c[c]	4.6ab	41d	161b
CWWM	8.9b	5.3a	60cd	180ab
PWWM	8.4bc	4.8ab	70bc	189ab
SWWS	10.1a	4.4b[d]	95a	211a
PWWC	10.4a	4.5b	46d	164b
A4PoW3	8.8b	5.4a[d]	82ab	221a

[a] Crop rotations are W, continuous winter wheat; CWWM, 3-yr rotation of corn-winter wheat-winter wheat-millet; PWWM, 3-yr rotation of pea-winter wheat-winter wheat-millet, SWWM, 3-yr rotation of sainfoin-winter wheat-winter wheat-sainfoin; PWWC, 4-yr rotation of pea-winter wheat-winter wheat-corn; and A4PoW3, 8-yr rotation of alfalfa (4 yr)-potato (1 yr)-winter wheat (3 yr).
[b] Total N input = Root biomass N + N fertilization rate + biologically fixed N (see Table 6).
[c] Numbers followed by different letters within a column of a parameter are significantly different at $P = 0.05$ by the least square means test.
[d] Includes only wheat grain yield.

concentration by the modified fumigation–incubation method for air-dried soils (Franzluebbers et al., 1996). The moist soil was fumigated with ethanol-free chloroform for 24 h and then incubated in a 1 l jar at 21 °C for another 10 d. After 10 d, NH_4–N and NO_3–N concentrations were determined as above after extracting the soil with KCl. The MBN concentration was calculated by the difference between the sum of NH_4–N and NO_3–N concentrations in the sample before and after fumigation–incubation and divided by a factor of 0.41 (Voroney and Paul, 1984). The NH_4–N and NO_3–N concentrations determined in the unfumigated-unincubated (or baseline) samples were used as available fractions of N. The concentrations of STN, PON, MBN, PNM, NH_4–N, and NO_3–N (g N kg^{-1} or mg N kg^{-1}) were converted into contents (Mg N ha^{-1} or kg N ha^{-1}) by multiplying their concentrations by the bulk density and the thickness of the soil layer and adjusting for equivalent soil mass as suggested by Lee et al. (2009). Total content at 0–30 cm was calculated by summing contents from individual depths. Soil N fractions for a crop rotation were determined by averaging N fractions under each crop within a rotation. Nitrogen sequestration rate at 0–30 cm was determined by deducting initial STN in 1984 from final STN in 2014 divided by 30 (number of years).

Nitrogen balance for a crop rotation was determined by deducting N removal through crop aboveground biomass and grain and N sequestration rate from total N inputs from N fertilization and biological N fixation (Sainju, 2017). Nitrogen inputs from atmospheric N deposition and crop seeds and N outputs from environmental N losses (N leaching, volatilization, denitrification, gaseous emissions, surface runoff, and plant senescence) are small (Sainju et al., 2016; Sainju, 2017) and were not considered for calculation of N balance.

2.4. Data analysis

Data for STN, PON, PNM, MBN, NH_4–N, and NO_3–N contents at a soil depth were analyzed using the MIXED model of SAS (Littell et al., 2006). Crop rotation was considered as the fixed effect and replication as the random effect. Data for crop biomass and grain yields and root biomass N were also analyzed as above where crop rotation and year were considered as fixed effects and replication as a random effect. Means were separated by using the least square means test when treatments and interactions were significant (Littell et al., 2006). When significant, linear regression and correlation analysis were conducted between soil N fractions, total N input, and length of the crop rotation. Multiple linear regression analysis was also conducted between total N input, crop rotation length, and soil N fractions at each depth to determine if their relationships can be improved. Statistical significance was evaluated at $P \leq 0.05$, unless otherwise stated. Because data on crop biomass and grain yields have been reported elsewhere (Guo et al., 2008; Cai and Hao, 2015), only annualized crop biomass and grain yields and estimated root biomass N content for each crop rotation (total yield or N content of all crops in the rotation divided by number of crops in a year), averaged across years, were reported for this study.

3. Results and discussion

3.1. Mean annualized crop biomass and grain yields and root biomass nitrogen

Mean annualized aboveground crop biomass (stems and leaves) yield across years was greater in SWWS and PWWC than other crop rotations, and greater in A4PoW3 and CWWM than W. In contrast, mean annualized crop grain yield was greater in CWWM and A4PoW3 than SWWS and PWWC. Estimated mean annualized root biomass N returned to the soil was greater in SWWS than W, CWWM, PWWM, and PWWC, and greater in A4PoW3 than W, CWWM, and PWWC (Table 3).

The greater annualized crop biomass yield in SWWS and PWWC was due to increased biomass of corn and sainfoin in the rotation. Biomass yield of corn and sainfoin averaged > 12 Mg ha^{-1}. Although corn was also present in CWWM, greater biomass yield in PWWC was probably a result of the presence of pea, a legume, which may have increased yields of succeeding crops by supplying N through N fixation as well as efficiently utilizing soil water (Guo et al., 2008; Cai and Hao, 2015). Besides supplying N, pea utilizes less soil water than wheat and barley due to early maturity, thereby leaving more water for the succeeding crops (Sainju et al., 2012; Lenssen et al., 2007, 2014). Continuous winter wheat (W), however, yielded less biomass than other crop rotations. Similarly, greater annualized grain yield in CWWM and A4PoW3 was due to increased grain yields of wheat and corn in these rotations. In contrast, lower grain yield in PWWC than CWWM was probably related to the length of the crop rotation. The length of the crop rotation was 4 yr in PWWC compared with 3 yr in CWWM (Table 1). Reduced cropping intensity (number of crops in a rotation divided by the rotation length) may have decreased grain yield in PWWC compared with CWWM. The trend in grain yield between PWWC and CWWM was in contrast to that with biomass yield, suggesting that grain and biomass yields may not follow similar trends. It may be possible that N supplied by pea residue increased biomass compared with grain yield of crops, more in PWWC than CWWM. Similar results have been reported by several researchers from the same experiment in the past (Guo et al., 2008; Cai and Hao, 2015).

The greater estimated annualized root biomass N returned to the soil in SWWS was a result of greater root/shoot ratio due to higher root biomass and N concentration in sainfoin (Table 2). Although N concentration was higher in alfalfa and potato biomass, lower root biomass yield of alfalfa compared with sainfoin and removal of potato tuber reduced root biomass N in A4PoW3. The lower root biomass N in PWWC than CWWM and PWWM was due to decreased root biomass yield as a result of reduced cropping intensity or increased length of the crop rotation (4 yr in PWWC compared with 3 yr in CWWM and PWWM). Decreased crop yield also reduced root biomass N in W.

3.2. Soil total nitrogen

Soil total N varied among crop rotations at various soil depths (Table 4). At 0–15 cm, STN was greater in CWWM than F, W, PWWM, and PWWC, and greater in SWWS, PWWC, and A4PoW3 than F, W, and PWWM. At 15–30 cm, STN was greater in A4PoW3 than other crop rotations, except CWWM and PWWM, and greater in CWWM, PWWM, SWWS, and PWWC than F and W. At 0–30 cm, STN was greater in A4PoW3 than other crop rotations, except CWWM and SWWS, and greater in CWWM, SWWS and PWWC than F and W. Increased total N input (root biomass N + N fertilization rate + biologically fixed N [Table 3]) returned to the soil linearly ($P \le 0.10$) increased STN at all depths (Fig. 1A). An increase in total N input by 1 kg N ha^{-1} yr^{-1} for 30 yr increased STN by 2 kg N ha^{-1} at 0–15 cm to 6 kg N ha^{-1} at 0–30 cm. The length of the crop rotation was also correlated with STN at 0–30 cm (Table 5). Multiple linear regression analysis of total N input and crop rotation length with STN improved R^2 values, which were significant at all depths (Table 6).

The lower STN in F than other crop rotations at all depths was likely a result of the absence of total N input and enhanced soil N mineralization due to increased soil temperature and water content during the fallow period. Various researchers (Peterson et al., 1998; Halvorson et al., 2002; Sainju et al., 2009) have reported that the absence of N input and increased N mineralization due to enhanced microbial activity as a result of higher soil temperature and water content reduces STN in the fallow treatment.

Reduction in total N input also appeared to reduce STN in W than other crop rotations, as total N input was linearly related with STN (Fig. 1A). Root biomass N and total N input were lower in W than other crop rotations (Table 3). In contrast, greater STN in CWWM, SWWS, and A4PoW3 was probably a result of increased root biomass N and total N input returned to the soil and/or higher C/N ratio of the residue. While root N and total N input were greater in SWWS and A4PoW3 than other crop rotations (Table 3), higher C/N ratio of corn residue than other crops may have increased STN in CWWM. Greater STN with increased crop residue N input have been reported by several researchers (Yang and Kay, 2001; Sainju and Lenssen, 2011; Sainju, 2013). Considering that crop residues in general have C concentrations of 400 g C kg^{-1} (Wood et al., 1990; Bollinder et al., 1997), C/N ratio of corn root biomass with N concentration of 8.3 g N kg^{-1} (Table 2) would be 48 compared with 40, 32, and 34 for wheat, pea, and millet root residues. The STN is influenced both by quantity and quality (C/N ratio) of crop residue returned to the soil (Kuo et al., 1997; Sainju et al., 2006, 2012). Crop residues with higher C/N ratios decompose more slowly than residues with lower ratios, thereby increasing STN (Kuo et al., 1997; Sainju et al., 2006, 2012). Although PWWC also contained corn in the rotation, lower root and total N input (Table 3) likely reduced STN in this rotation compared with CWWM. It is likely that corn residue added every 3 yr increased STN in CWWM due to increased root N input compared with residue added every 4 yr in PWWC.

Our results indicate that the presence of legume forages for a longer period in the crop rotation, such as in A4PoW3 and SWWS, increased STN compared with other rotations. Increased N inputs, followed by reduced soil organic N mineralization due to relatively undisturbed soil condition appeared to increase STN in these rotations. Increased STN with alfalfa compared with annual crops has been previously reported (Sainju and Lenssen, 2011). Strong correlation between the length of crop rotation and STN (Table 5) as well as improved relationship using multiple linear regression of total N input and crop rotation length with STN at all depths (Table 6) indicate that both increased total N input and crop rotation length promoted soil N storage. Enhanced crop rotation provides other benefits, such as increased water- and N-use efficiency and reduced infestations of weeds, diseases, and pests which help to increase crop yield compared with monocropping (Karlen et al., 1994; Yusuf et al., 2009; Lenssen et al., 2007, 2014) As a result, diversified crop rotation increases STN compared with monocropping due to increased total N input (Sainju et al., 2012, 2016).

Table 4
Soil total nitrogen (STN) at 0–15, 15–30, and 0–30 cm depths as affected by crop rotation.

Crop rotation[a]	STN (Mg N ha^{-1})		
	0-15 cm	15-30 cm	0-30 cm
F	2.03 ± 0.16c[b]	1.88 ± 0.14d	3.91 ± 0.27e
W	2.20 ± 0.13c	2.16 ± 0.13c	4.36 ± 0.02d
CWWM	2.70 ± 0.15a	2.61 ± 0.08ab	5.31 ± 0.19ab
PWWM	2.16 ± 0.12c	2.52 ± 0.29ab	4.68 ± 0.40 cd
SWWS	2.59 ± 0.08ab	2.46 ± 0.12b	5.05 ± 0.15abc
PWWC	2.46 ± 0.02b	2.48 ± 0.07b	4.94 ± 0.06bc
A4PoW3	2.63 ± 0.07ab	2.78 ± 0.13a	5.40 ± 0.15a

[a] Crop rotations are W, continuous winter wheat; CWWM, 3-yr rotation of corn-winter wheat-winter wheat-millet; PWWM, 3-yr rotation of pea-winter wheat-winter wheat-millet, SWWM, 3-yr rotation of sainfoin-winter wheat-winter wheat-sainfoin; PWWC, 4-yr rotation of pea-winter wheat-winter wheat-corn; and A4PoW3, 8-yr rotation of alfalfa (4 yr)-potato (1 yr)-winter wheat (3 yr).
[b] Numbers followed by different letters within a column of a parameter are significantly different at $P = 0.05$ by the least square means test.

3.3. Particulate organic nitrogen

Similar to STN, PON at all depths varied with crop rotations (Table 7). At 0–15 cm, PON was greater with CWWM than other crop rotations, except A4PoW3, and greater in A4PoW3 than F, W and PWWM. At 15–30 cm, PON was greater in PWWC than F, W, PWWM, and A4PoW3. At 0–30 cm, PON was greater in CWWM than F, W, and PWWM. Total N input was linearly related to PON at 0–30 cm ($P \le 0.10$) (Fig. 1B). The length of the crop rotation was weakly correlated with PON at 0–30 cm (Table 5).

Reduced or no total N input may have decreased PON in F and W compared with other crop rotations, a case similar to that observed for STN. The greater PON in CWWM and PWWC than other rotations could be a result of inclusion of crops, such as corn, with higher C/N ratio in the rotation. It could be possible that coarse fragments of corn root residue decompose more slowly due to higher C/N ratio (48 in corn compared with 32–40 in other crops) than other crops, resulting in greater PON in CWWM and PWWC. In contrast, increased total N input probably increased PON in SWWS and A4PoW3. Several researchers

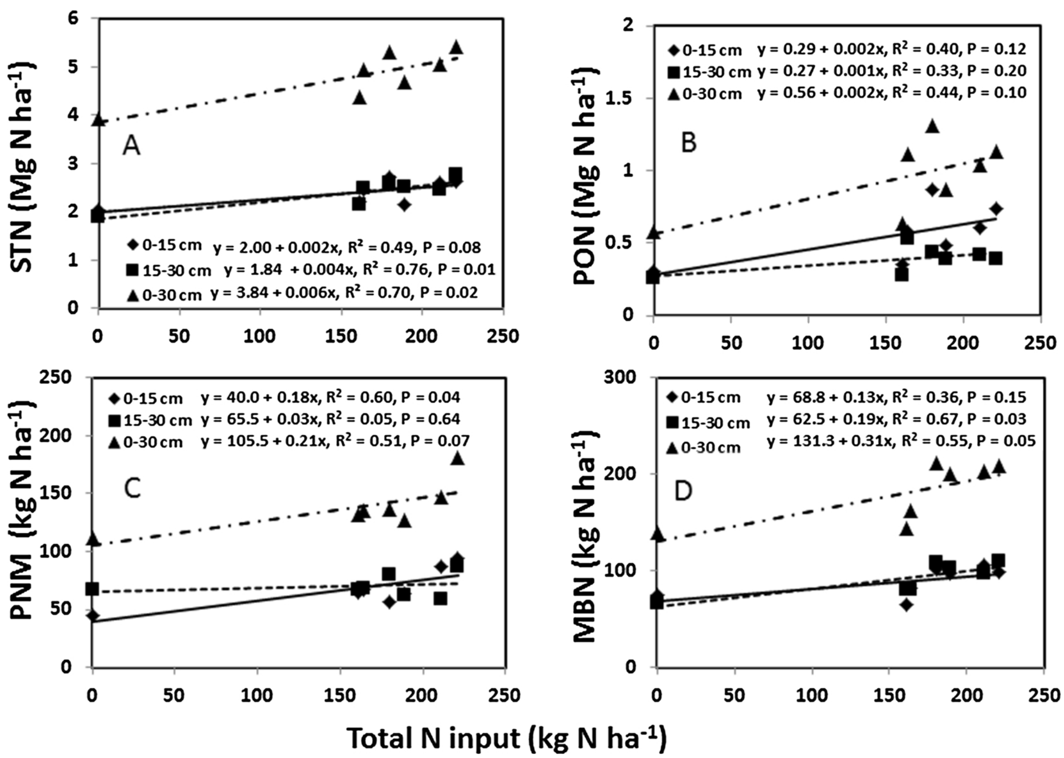

Fig. 1. Relationships between mean annualized total N input and soil N fractions at 0–15, 15–30, and 0–30 cm depths. Soil N fractions are MBN, microbial biomass N; PNM, potential nitrogen mineralization; PON, particular organic nitrogen; and STN, soil total nitrogen. Solid (—), dashed (———), and dashed-dotted (-·-·) lines denote regressions lines for 0–15, 15–30, and 0–30 cm, respectively.

Table 5
Correlation (r) between the length of the crop rotation and soil N fractions [a] at the 0–30 cm depth (n = 7).

Parameter	Rotation length	STN	PON	PNM	MBN	NH_4-N	NO_3-N
Rotation length	1.00	0.82*	0.68†	0.92**	0.65†	−0.53	0.24
STN		1.00	0.94**	0.78*	0.85*	−0.81*	0.60
PON			1.00	0.57	0.79*	−0.73†	0.79*
PNM				1.00	0.61	−0.56	0.09
MBN					1.00	−0.91**	0.57
NH_4-N						1.00	−0.64
NO_3-N							1.00

† Significant at $P \leq 0.10$.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
[a] Soil N fractions are MBN, microbial biomass N; PNM, potential nitrogen mineralization; PON, particulate organic nitrogen; and STN, soil total nitrogen.

Table 6
Multiple linear regression analysis of total N input and length of the crop rotation with soil N fractions at the 0–30 cm depth.

Soil depth	Soil N fraction[a]	Intercept	Total N input[b]	Rotation length	R^2	*P*
0-15 cm	STN	2.02	0.002	0.04	0.56	0.07
	PON	0.31	0.001	0.04	0.53	0.22
	PNM	41.81	0.11	2.84	0.70	0.09
	MBN	70.00	0.08	1.85	0.41	0.35
15-30 cm	STN	1.88	0.002	0.06	0.92	0.01
	PON	0.27	0.001	0.01	0.39	0.37
	PNM	67.82	−0.06	3.59	0.51	0.24
	MBN	63.61	0.14	1.81	0.71	0.09
0-30 cm	STN	3.91	0.003	0.10	0.82	0.03
	PON	0.60	0.001	0.05	0.55	0.20
	PNM	109.66	0.04	6.42	0.82	0.03
	MBN	133.61	0.22	3.67	0.60	0.16

[a] Soil N fractions are MBN, microbial biomass N; PNM, potential nitrogen mineralization; PON, particulate organic nitrogen; and STN, soil total nitrogen.
[b] Total N input = Root biomass N + N fertilization rate + biologically fixed N.

(Moore et al., 2000; Sainju and Lenssen, 2011) have reported that crop rotations containing alfalfa alone or in rotation with cereal crops had greater PON than annual crops.

The PON/STN ratio at 0–15 cm was greater in CWWM and A4PoW3 than other crop rotations (Table 7). At 15–30 cm, the PON/STN ratio was greater in PWWC than F, W, PWWM, and A4PoW3. At 0–30 cm, the PON/STN ratio was greater in CWWM than F, W, and PWWM. The PON/STN ratio was greater at 0–15 than at 15–30 cm for all crop rotations. The proportion of PON in STN varied from 130 g kg^{-1} STN in W at 15–30 cm to 320 g kg^{-1} STN in CWWM at 0–15 cm.

The PON/STN ratio measures N in coarse organic matter fraction relative to soil total N. As PON mineralize more rapidly than STN, soils with greater PON/STN ratio have higher proportion of soil organic matter that is mineralized rapidly compared with soils with lower ratio. The greater PON/STN ratio in CWWM and PWWC was probably a result of higher proportion of N in coarse organic matter relative to STN in corn residue that decomposed more slowly than other crop residues due to larger C/N ratio. Increased coarse organic N relative to STN also appeared to increase the PON/STN ratio in A4PoW3. Reduction in PON clearly reduced the PON/STN ratios in F, W, and PWWM. Higher N input due to increased root biomass at the soil surface likely increased the PON/STN ratio at 0–15 than at 15–30 cm. The PON/STN ratio at 0–30 cm observed in this experiment (144–244 g kg^{-1} STN) was similar to the ratio at 0–30 cm (175-238 g kg^{-1} STN) reported in the northern Great Plains, USA (Sainju et al., 2009; Sainju and Lenssen, 2011).

Table 7
Soil particulate organic nitrogen (PON), potential N mineralization (PNM), PON/soil total nitrogen (STN) ratio, and PNM/STN ratio at 0–15, 15–30, and 0–30 cm depths as affected by crop rotation.

Crop rotation[a]	PON (Mg N ha^{-1})			PON/STN ratio (g kg^{-1} STN)		
	0-15 cm	15-30 cm	0-30 cm	0-15 cm	15-30 cm	0-30 cm
F	0.31 ± 0.03e[b]	0.26 ± 0.02c	0.58 ± 0.05d	155 ± 4.30c	140 ± 3.57b	147 ± 2.58c
W	0.35 ± 0.08de	0.28 ± 0.07c	0.63 ± 0.12 cd	158 ± 34.7c	130 ± 29.7b	144 ± 29.5c
CWWM	0.87 ± 0.23a	0.44 ± 0.04ab	1.31a ± 0.27	320 ± 69.7a	167 ± 15.2ab	244 ± 42.4a
PWWM	0.48 ± 0.12cde	0.39 ± 0.04b	0.87bc ± 0.14	219 ± 44.8bc	158 ± 25.8b	188 ± 23.1bc
SWWS	0.61 ± 0.17bc	0.42 ± 0.05ab	1.04ab ± 0.13	235 ± 57.8bc	174 ± 25.9ab	204 ± 25.5ab
PWWC	0.58 ± 0.04bcd	0.53 ± 0.10a	1.11ab ± 0.14	235 ± 16.1bc	214 ± 44.2a	224 ± 29.2ab
A4PoW3	0.74 ± 0.10ab	0.39 ± 0.04b	1.13ab ± 0.10	283 ± 45.2a	139 ± 8.54b	211 ± 22.7ab
Crop rotation[a]	**PNM (kg N ha^{-1})**			**PNM/STN ratio (g kg^{-1} STN)**		
	0-15 cm	15-30 cm	0-30 cm	0-15 cm	15-30 cm	0-30 cm
F	45.1 ± 4.30d	67.0 ± 5.41b	112.1 ± 9.71d	22.5 ± 3.89d	35.7 ± 4.04a	29.1 ± 3.83bc
W	65.1 ± 4.22bc	66.9 ± 7.57b	132.0 ± 3.58c	29.6 ± 1.18bc	30.9 ± 1.76ab	30.3 ± 0.74ab
CWWM	56.3 ± 1.79c	80.3 ± 2.57a	136.6 ± 3.57bc	20.9 ± 1.66d	30.8 ± 0.44ab	25.8 ± 0.61c
PWWM	63.9 ± 2.17bc	62.8 ± 2.37b	126.7 ± 4.53c	29.7 ± 2.58bc	25.2 ± 3.73c	27.5 ± 3.15bc
SWWS	87.2 ± 4.98a	59.4 ± 0.28b	146.6 ± 4.91b	33.7 ± 2.52ab	24.2 ± 1.13c	29.0 ± 1.77bc
PWWC	67.6 ± 8.41b	68.1 ± 4.12b	135.7 ± 7.31bc	27.4 ± 3.55c	27.5 ± 2.26bc	27.5 ± 1.30bc
A4PoW3	94.2 ± 6.19a	87.4 ± 12.4a	181.3 ± 12.2a	35.8 ± 1.61a	31.4 ± 2.99ab	33.6 ± 1.34a

[a] Crop rotations are W, continuous winter wheat; CWWM, 3-yr rotation of corn-winter wheat-winter wheat-millet; PWWM, 3-yr rotation of pea-winter wheat-winter wheat-millet, SWWM, 3-yr rotation of sainfoin-winter wheat-winter wheat-sainfoin; PWWC, 4-yr rotation of pea-winter wheat-winter wheat-corn; and A4PoW3, 8-yr rotation of alfalfa (4 yr)-potato (1 yr)-winter wheat (3 yr).
[b] Numbers followed by different letters within a column of a parameter are significantly different at $P = 0.05$ by the least square means test.

3.4. Potential nitrogen mineralization

Differences in crop rotations resulted in variations in PNM at all depths (Table 7). At 0–15 cm, PNM was greater in SWWS and A4PoW3 than other crop rotations and greater in PWWC than F and CWWM. At 15–30 cm, PNM was greater in CWWM and A4PoW3 than other crop rotations. At 0–30 cm, PNM was greater in A4PoW3 than other crop rotations, and greater in SWWS than F, W, and PWWM. Increase in total N input linearly increased PNM at 0–15 and 0–30 cm (Fig. 1C). The length of the crop rotation was also strongly correlated with PNM at 0–30 cm (Table 6). Inclusion of crop rotation length with total N input in a multiple linear regression further increased the relationship with PNM at 0–15 and 0–30 cm (Table 6).

The greater PNM in SWWS and A4PoW3 than other crop rotations was probably a result of increased total N input to the soil (Table 3), followed by rapid mineralization of the alfalfa and sainfoin root residue due to their lower C/N ratios than other crops (Kuo et al., 1997; Sainju and Lenssen, 2011). With estimated C concentration of 400 g kg^{-1} each (Wood et al., 1990; Bollinder et al., 1997) and N concentrations of 13.1 and 14.8 g N kg for sainfoin and alfalfa root residue (Table 2), the C/N ratios of sainfoin and alfalfa roots were 31 and 27, respectively, compared with 34–48 for annual crop roots. Residues with higher N concentrations or lower C/N ratios can mineralize rapidly compared with those with lower ratios, which can increase PNM (Sainju and Lenssen, 2011). Greater PNM with alfalfa and legume forages than cereal crops and nonlegumes have been reported before (Sainju et al., 2006; Sainju and Lenssen, 2011). Inclusion of legume forages, such as alfalfa and sainfoin, for a longer period, thereby increasing the length of the crop rotation, appeared to favor PNM. Reduction or no total N input may have reduced PNM in F and W. As with STN, both total N input and crop rotation length further favored PNM at 0–15 and 0–30 cm compared with either factor alone, indicating enhanced soil N mineralization with increased N input and crop rotation length.

The PNM/STN ratio indicates the proportion of soil total N that is mineralizable during a crop growing season. The greater the ratio, the higher is the mineralization potential of STN. The PNM/STN ratio at 0–15 cm was greater in A4PoW3 than other crop rotations, except SWWS, and greater in SWWS than F, W, CWWM, and PWWC (Table 7). At 15–30 cm, the PNM/STN ratio was greater in F than PWWM, SWWS, and PWWC. At 0–30 cm, the PNM/STN ratio was greater in A4PoW3 than other crop rotations, except W, and greater in W than CWWM. The PNM/STN ratio ranged from 20.9 g kg^{-1} STN for PWWM to 35.8 g kg^{-1} STN for A4PoW3 at 0–15 cm.

Increased PNM relative to STN at 0–15 and 0–30 cm increased the PNM/STN ratio in A4PoW3, probably a result of inclusion of alfalfa in the crop rotation for a longer period. Increased microbial activity due to enhanced soil temperature and water content during the fallow period likely increased N mineralization relative to soil N storage (Peterson et al., 1998; Halvorson et al., 2002) and therefore the PNM/STN ratio in F at 15–30 cm (Sainju et al., 2009). Our PNM/STN ratios of 20.9-35.8 g kg^{-1} STN at 0–15 and 15–30 cm were higher than 8.4-24.7 g kg^{-1} STN at the same depths in the northern Great Plains, USA (Sainju and Lenssen, 2011). Differences in soil and climatic conditions may have resulted in variations in PNM/STN ratios among regions. Soil texture in our experiment was silt loam compared with loam in the northern Great Plains, USA. Average air temperature is 9.1 °C and annual precipitation 540 mm in our experimental site compared with air temperature of 5.4 °C and annual precipitation of 340 mm in the northern Great Plains, USA. Higher air temperature and precipitation may have increased N mineralization relative to soil N storage and therefore the PNM/STN ratio in our site compared with that in the northern Great Plains, USA.

3.5. Microbial biomass nitrogen

Similar to other N fractions, MBN at all depths varied with crop rotations (Table 8). At 0–15, 15–30 cm, and 0–30 cm, MBN was greater in CWWM, PWWM, SWWS, and A4PoW3 than other crop rotations. The MBN was greater in F than W at 0–15 cm, but the trend reversed at 15–30 cm. Increase in total N input linearly increased MBN at 15–30 and 0–30 cm (Fig. 1D). An increase in root biomass N input by 1 kg N ha^{-1} yr^{-1} for 30 yr increased MBN by from 0.19 kg N ha^{-1} at 15–30 cm to 0.31 kg N ha^{-1} at 0–30 cm. The length of the crop rotation was weakly correlated with STN at 0–30 cm (Table 5). Multiple linear regression of total N input with crop rotation length did not improve their relationships with MBN at all depths (Table 6).

Increased total N input and/or higher C/N ratio of crop residue in

Table 8
Soil microbial biomass N (MBN), MBN/soil total nitrogen (STN) ratio, and NH_4-N and NO_3-N contents at 0–15, 15–30, and 0–30 cm depths as affected by crop rotation.

Crop rotation[a]	MBN (kg N ha^{-1})			MBN/STN ratio (g kg^{-1} STN)		
	0-15 cm	15-30 cm	0-30 cm	0-15 cm	15-30 cm	0-30 cm
F	74.5 ± 6.13b[b]	65.9 ± 8.18c	140.4 ± 13.7b	36.7 ± 0.18bc	35.0 ± 2.17bc	35.8 ± 1.13bc
W	64.5 ± 6.21c	79.9 ± 6.73b	144.4 ± 12.7b	29.3 ± 2.68d	37.1 ± 3.83abc	33.2 ± 2.90c
CWWM	102.0 ± 3.30a	109.0 ± 5.64a	211.0 ± 8.76a	37.9 ± 1.90bc	41.9 ± 3.04a	39.9 ± 1.90ab
PWWM	96.9 ± 1.79a	103.0 ± 14.7a	199.9 ± 15.1a	44.9 ± 3.16a	41.6 ± 7.06ab	43.3 ± 4.48a
SWWS	106.0 ± 14.3a	97.5 ± 0.88a	203.5 ± 15.2a	41.3 ± 6.61ab	39.8 ± 1.91ab	40.5 ± 3.36ab
PWWC	81.6 ± 12.4b	80.9 ± 3.72b	162.5 ± 15.6b	33.1 ± 5.08 cd	32.6 ± 1.31c	32.9 ± 2.78c
A4PoW3	98.6 ± 4.29a	110.0 ± 7.68a	208.6 ± 3.54a	37.5 ± 1.55bc	39.6 ± 1.02ab	38.6 ± 0.57ab
Crop rotation[a]	NH_4-N (kg N ha^{-1})			NO_3-N (kg N ha^{-1})		
	0-15 cm	15-30 cm	0-30 cm	0-15 cm	15-30 cm	0-30 cm
F	34.7 ± 0.05a	44.6 ± 0.10a	79.3 ± 0.04a	21.0 ± 2.25b	10.9 ± 1.50b	31.9 ± 1.49b
W	33.2 ± 1.84ab	38.9 ± 1.66b	72.1 ± 3.03b	13.6 ± 3.52c	15.5 ± 2.57ab	29.1 ± 6.09b
CWWM	25.6 ± 0.16c	32.0 ± 1.02d	57.6 ± 1.18d	29.8 ± 1.55a	16.2 ± 2.48ab	46.0 ± 1.45a
PWWM	30.5 ± 0.83b	35.4 ± 1.81c	65.9 ± 2.63c	17.4 ± 1.04bc	16.7 ± 4.27a	34.1 ± 4.09b
SWWS	31.5 ± 3.16b	34.4 ± 1.07 cd	65.9 ± 2.78c	18.1 ± 0.65bc	14.5 ± 2.92ab	32.6 ± 2.27b
PWWC	34.3 ± 0.14a	41.0 ± 2.55b	75.3 ± 2.68b	19.8 ± 4.07b	16.2 ± 3.76ab	36.0 ± 7.74b
A4PoW3	30.7 ± 0.52b	32.0 ± 1.77d	62.7 ± 1.57c	18.6 ± 1.85b	15.9 ± 1.23ab	34.5 ± 2.62b

[a] Crop rotations are W, continuous winter wheat; CWWM, 3-yr rotation of corn-winter wheat-winter wheat-millet; PWWM, 3-yr rotation of pea-winter wheat-winter wheat-millet, SWWM, 3-yr rotation of sainfoin-winter wheat-winter wheat-sainfoin; PWWC, 4-yr rotation of pea-winter wheat-winter wheat-corn; and A4PoW3, 8-yr rotation of alfalfa (4 yr)-potato (1 yr)-winter wheat (3 yr).

[b] Numbers followed by different letters within a column of a parameter are significantly different at $P = 0.05$ by the least square means test.

the rotation appeared to increase MBN in CWWM, PWWM, SWWS, and A4PoW3. Total N input was higher in these rotations than W (Table 3). Several researchers (Balota et al., 2003; Yusuf et al., 2009) have found greater MBN in legume-based crop rotations than continuous corn and fallow due to higher crop residue N input. Crop residues with higher C/N ratios, such as corn, wheat, and millet, may have increased MBN in CWWM, as residues with higher C/N ratios can immobilize more soil N than residues with low ratios. The MBN represents N immobilized by the microbial biomass where N becomes temporarily unavailable to crops. This also holds true for greater MBN at 15–30 cm in W than F. The reasons for greater MBN in F than W at 0–15 cm were not known. Reduced total N input and decreased diversity of crop residues, however, may have reduced MBN in F and W compared with other crop rotations (Angers et al., 1993; Moore et al., 2000). The lower MBN in PWWC than CWWM, PWWM, and SWWS was probably related to total N input and the length of the crop rotation. Total N input was lower (Table 3) and the length of the crop rotation was 4 yr for PWWC compared with 3 yr for CWWM, PWWM, and SWWS (Table 1). Decreased N immobilization due to lower total N input and cropping intensity likely reduced MBN in PWWC than other 3-yr rotations.

In contrast to the PNM/STN ratio, the MBN/STN ratio measures N immobilization potential of the soil relative to STN. The MBN/STN ratio at 0–15 cm was greater in PWWM than other crop rotations, except SWWS (Table 8). At 15–30 cm, the MBN/STN ratio was greater in CWWM than F and PWWC. At 0–30 cm, the MBN/STN ratio was greater in PWWM than F, W, and PWWC. The MBN/STN ratio varied from 29.3 g kg^{-1} STN in W to 44.9 g kg^{-1} STN in PWWM at 0–15 cm.

The greater MBN/STN ratio in CWWM and PWWM suggests that a higher proportion of N was immobilized in microbial biomass relative to total N in these treatments. Microbial communities can develop a complex system of substrate-use efficiency with the heterogeneous input of organic matter, enabling them to fix a greater proportion of N in their biomass relative to STN in crop rotations than monocropping (Moore et al., 2000). Our MBN/STN ratios were similar to 25.0-55.3 g kg^{-1} STN reported by Yusuf et al (2009), but lower than 39.4 to 85.8 g kg^{-1} STN observed by Sainju and Lenssen (2011) in the northern Great Plains, USA. Reduced N immobilization relative to soil N storage could have resulted in lower MBN/STN ratios in our site compared with ratios in the northern Great Plains, USA.

3.6. Soil ammonium- and nitrate-nitrogen

Soil NH_4-N content at 0–15 cm was greater in F and PWWC than other crop rotations, except W, and greater in W, PWWM, SWWS, and A4PoW3 than CWWM (Table 8). At 15–30 and 0–30 cm, NH_4-N content was also greater with F than other crop rotations and greater in W and PWWC than CWWM, PWWM, SWWS, and A4PoW3. Increased mineralization of soil organic N due to enhanced soil temperature and water content during the fallow period and lack of N uptake by crops may have increased NH_4-N content in F. While N supplied by legume residues and N fertilization may have maintained NH_4-N content in W, PWWM, SWWS, PWWC, and A4PoW3, lower NH_4-N content in CWWM could be a result of increased N uptake by nonlegume crops.

Soil NO_3-N content at 0–15 cm was greater in CWWM than other crop rotations and greater in F, PWWC, and A4PoW3 than W (Table 8). At 15–30 cm, NO_3-N content was greater in PWWM than F. At 0–30 cm, NO_3-N content was greater in CWWM than other crop rotations. Increased N fertilization rates to nonlegume crops and N fixed by pea residue probably increased NO_3-N content in CWWM and PWWM. Unlike NH_4-N content, the fallow treatment did not increase NO_3-N content compared with other treatments probably because NO_3-N can be lost to the environment through surface runoff, leaching, denitrification, and gaseous emissions.

3.7. Correlations among soil N fractions

At 0–30 cm, STN was positively correlated with PON, PNM, and MBN and negatively with NH_4-N (Table 5). The PON was positively correlated with MBN and NO_3-N and negatively with NH_4-N. The MBN was negatively correlated with NH_4-N. It has been well known that STN changes slowly over time due to soil and crop management practices (Franzluebbers et al., 1995; Sainju et al., 2009). Because of significant correlations, labile fractions, such as PON, PNM, and MBN may be used as indicators of STN changes during short period, such as within a crop growing season, when STN does not readily change.

Table 9
Nitrogen balance based on N inputs (N fertilization and biological N fixation), N outputs (crop biomass and grain N removal), and soil N sequestration rate at the 0–30 cm depth as affected by crop rotation.

Crop rotation[a]	N fertilization rate (kg N ha^{-1} yr^{-1}) (A)	Biological N fixation (kg N ha^{-1} yr^{-1}) (B)	Crop N removal (kg N ha^{-1} yr^{-1}) (C)	N sequestration rate (0-30 cm) (kg N ha^{-1} yr^{-1}) (D)	N balance[b] (kg N ha^{-1} yr^{-1})
F	0	0	0	−10	10
W	120	0	193	5	−78
CWWM	120	0	244	37	−161
PWWM	93	26	265	16	−162
SWWS	65	51	206	28	−118
PWWC	93	25	265	25	−172
A4PoW3	65	74	195	40	−96

[a] Crop rotations are W, continuous winter wheat; CWWM, 3-yr rotation of corn-winter wheat-winter wheat-millet; PWWM, 3-yr rotation of pea-winter wheat-winter wheat-millet, SWWM, 3-yr rotation of sainfoin-winter wheat-winter wheat-sainfoin; PWWC, 4-yr rotation of pea-winter wheat-winter wheat-corn; and A4PoW3, 8-yr rotation of alfalfa (4 yr)-potato (1 yr)-winter wheat (3 yr).
[b] N balance = Column (A) + Column (B) - Column (C) - Column (D).

3.8. Nitrogen balance

Nitrogen fertilization rate was higher in W and CWWM than other crop rotations (Table 9) because of increased N rates to nonlegume crops. Biological N fixation by pea, sainfoin, and alfalfa reduced N fertilization rates in PWWM, SWWS, and A4PoW3. Increased root biomass yield and N concentration in sainfoin and alfalfa compared with pea (Table 2), followed by their longer duration in the crop rotation, increased biological N fixation in SWWS and A4PoW3. Crop N removal in biomass and grain was greater in CWWM, PWWM, and PWWC than other crop rotations. Nitrogen sequestration rate at 0–30 cm varied from -10 kg N ha^{-1} yr^{-1} in F to 40 kg N ha^{-1} yr^{-1} in A4PoW3. Lack of N input resulted in negative N sequestration rate in F, but positive in other crop rotations. Nitrogen balance as the difference between N input (N fertilization rate + biological N fixation), N output (crop N removal), and N sequestration rate was positive in F, but negative in all other treatments (Table 9). Nitrogen balance was lower in crop rotations than W. Increased crop N removal and N sequestration rate reduced N balance with crop rotations compared with monocropping. This suggests that diversified crop rotations can enhance soil N storage and be more productive by removing more N from the soil, resulting in reduced N balance than monocropping and fallow.

4. Conclusions

Soil N fractions varied with long-term (30 yr) crop rotations due to variations in root biomass N returned to the soil, biological N fixation, N fertilization rate to crops, and C/N ratio of the root residue. Rotations containing legume perennial forages increased STN, PON, PNM, and MBN compared with monocropping and fallow due to increased total N input. Rotations containing crops with higher C/N ratio, such as corn, increased STN, PON, and MBN. While the fallow treatment had greater NH_4-N content, higher N fertilization rate to nonlegume crops had greater NO_3-N content in CWWM than other crop rotations. Soil N fractions were linearly related with total N input and also correlated with the length of the crop rotation. Combination of total N input and crop rotation length in multiple linear regression analysis further enhanced soil N storage and N mineralization. Nitrogen balance was lower with crop rotations than monocropping and fallow. Diversified crop rotations, such as CWWM, PWWM, SWWS, and A4PoW3, can increase soil N storage, mineralization, and availability due to increased total N input and more productive than monocropping and fallow. As a result, such rotations can maintain long-term soil fertility and sustainability of dryland cropping systems and reduce N fertilization rates to crops in the Loess Plateau of China.

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