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Fertilization regimes affect the soil biological characteristics of a sudangrass and ryegrass rotation system

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The sudangrass (*Sorghum sudanense*) and ryegrass (*Lolium multiflorum* L.) rotation is an intensive and new cropping system in Central China. Nutrient management practices in this rotation system may influence soil fertility, the important aspects of which are soil biological properties and quality. As sensitive soil biological properties and quality indicators, soil microbial community activity, microbial biomass, enzyme activities, soil organic matter (SOM) and total N resulting from different fertilization regimes in this rotation system were studied through a four-year field experiment from April 2005 to May 2009. Treatments included control (CK), fertilizer phosphorus and potassium (PK), fertilizer nitrogen and potassium (NK), fertilizer nitrogen and phosphorus (NP) and a fertilizer nitrogen, phosphorus and potassium combination (NPK). Soil microbial community activities in the NK, NP and NPK treatments were significantly lower than those in the CK and PK treatments after the sudangrass and ryegrass trial. The highest microbial biomass C, microbial biomass N, SOM, total N, sucrase and urease activities were found in the NPK treatment, and these soil quality indicators were significantly higher in the NK, NP and NPK treatments than in the PK and CK treatments. Soil microbial biomass and enzyme activities were positively associated with SOM in the sudangrass and ryegrass rotation system, indicating that fertilization regimes, especially N application, reduced microbial community activity in the soil. Proper fertilization regimes will increase microbial biomass, enzyme activity and SOM and improve soil fertility.

microbial activity, microbial biomass carbon and nitrogen, enzyme, fertilization regimes

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With increasing population, intensive cultivation is necessary to produce more food from limited farmland [1,2]. One particularly serious problem is the decrease in organic matter content of agricultural soils, which may endanger soil fertility [3]. Fertilizers have been widely recommended for sustainable agricultural production in the world, including China [2,3]. Because of fertilizer application and crop growth, the structure of agroecosystems is also altered [4,5].

Fertile soils provide essential nutrients for crop growth and have good soil structure [6]. Microbial community activity, microbial biomass and enzymes are all soil quality indicators in agroecosystems [7]. Microbial community activity is an important indicator of microbial function in the soil and can easily be affected by anthropogenic disturbances such as fertilization regimes [8]. Fertilizers, especially nitrogen (N), phosphorus (P), and potassium (K) may directly or indirectly induce changes in soil biological characteristics [9]. Katayama *et al*. [4] have reported that fertilizer application has a direct effect on the composition of the soil microbial community in monocultures and fallow soil. Some evidence also suggests that soil microbial community activity is indirectly affected by changes in plant commu-

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nity composition resulting from fertilizer regimes [10,11]. Microbial biomass also has a critical role in the soil, regulating processes such as organic matter decomposition and nutrient cycling [12]. Enzyme activities are associated with the microbial biomass [13,14]. Enzymes have a crucial role in the cycling of nutrients such as carbon (C) and N, and fertilization regimes can also increase soil enzymatic activities in agricultural systems [15]. Organic matter and total N are important chemical quality indicators in the soil. Fertilization regimes directly or indirectly influence soil organic matter (SOM) and total N [16,17].

Sudangrass (*Sorghum sudanense*, a C_4 species) and ryegrass (*Lolium multiflorum* L., a C_3 species) are graminaceous forage grasses which are important for livestock farming and fisheries in China. In recent years, because of the rapid development of forage grasses with the sufficient light and abundant rainfall in the south of China, the sudangrass and ryegrass rotation system has developed as a new type of cropping system [18]. In this rotation, forage grasses are harvested eight or nine times every year, and because of its higher frequency and intensity, nutrient uptake induced by the greater amounts of aboveground biomass is much higher than that of common farm crops [18,19]. As an intensive and new cropping system, it is essential to develop proper nutrient management measures for crop growth requirement using fertilization regimes that are sustainable and healthy for agroecosystems. The objectives of this research were (i) to study the effect of fertilization regimes on microbial characteristics such as community activity, microbial biomass C (MBC) and microbial biomass N (MBN), enzyme activities, SOM and total N in soil, and (ii) to investigate proper fertilization measures for sustainable soil fertility and agricultural production for the increasing population.

1 Materials and methods

1.1 Description of the study site

The field experiment was carried out on the Agricultural Research Station of Datonghu Administration District, Jingzhou, Hubei Province, China (30°3′N, 113°45′E). The experimental soil is classified as a fluvo-aquic soil with a soil pH of 6.93 (1:2.5 water: soil), 18.5 g organic matter kg^{-1}, 1.05 g total N kg^{-1}, 12.0 mg available P kg^{-1}, and 121.7 mg available K kg^{-1}. The climate is warm and moist, and the average annual precipitation is around 1200 mm.

1.2 Experimental materials and design

The four-year rotation experiment incorporating planting sudangrass (*Sorghum sudanense* cv. Yanchi) in summer and ryegrass (*Lolium multiflorum* cv. Abundant) in winter was conducted in Jianghan Plain from April 2005 to May 2009. Sudangrass and ryegrass seeds were directly broadcast sown in each plot (7.5 m×2 m), and the two forage grasses were harvested eight times a year.

The experimental design consisted of five treatments in a randomized block design with four replicates, including control (CK), fertilizer phosphorus and potassium (PK), fertilizer nitrogen and potassium (NK), fertilizer nitrogen and phosphorus (NP) and a fertilizer nitrogen, phosphorus and potassium combination (NPK). Based on our previous research [18,19], N, P, and K were applied at rates of 450 kg N hm^{-2}, 180 kg P_2O_5 hm^{-2} and 300 kg K_2O hm^{-2} in the sudangrass trial, with one third of the N and half of the K applied as a basal dressing and the remaining nutrients applied in three applications by topdressing. In the ryegrass trial, total nutrient application rates were 225 kg N hm^{-2}, 135 kg P_2O_5 hm^{-2} and 150 kg K_2O hm^{-2}. Half of the N and K were applied as a basal dressing and the remaining nutrients by topdressing in two applications. Urea, calcium superphosphate and potassium chloride were used as the sources of fertilizer N, P and K, respectively.

1.3 Soil sampling and assay

The original soil was sampled from the upper layer (0–20 cm) in the field before the trial began. After both sudangrass and ryegrass were harvested in the final year of the study from 2008 to 2009, fresh soil samples were also collected from the upper layer (0–20 cm) in each plot. The samples were carefully passed through a 2-mm sieve for the analysis of soil microbial community activity, biomass and enzymes. To determine SOM and total N, the soil samples were air-dried and sieved through a 0.149-mm mesh.

Microbial community activity was analyzed by developing community physiological profiles using Biolog assays [20,21]. Briefly, 10 g of sieved soil was extracted in 100 mL of sterile saline solution (0.85%, m/v) with 5 g glass beads on a rotary shaker at 200 r min^{-1} for 30 min at 25°C. Suspensions were diluted 100-fold with sterile water and 150 μL of the dilution was added to each well of a Biolog Eco-plate™. The plates were scanned at 590 nm at 24 h intervals for 7 d, using an automated Biolog micro-station (Biolog, Hayward, CA, USA).

MBC and MBN were measured by the fumigation-extraction method [22]. Soil sucrase and urease were measured by the method reported by Guan [23]. SOM was determined by the oxidation method using $K_2Cr_2O_7$ and concentrated sulfuric acid at 170–180°C, and total N by the regular Kjeldhal method [24].

1.4 Data analysis

The average well color development (AWCD) in the Biolog Eco-plate was calculated according to Garland [20], and Biolog data were interpreted by principal component analysis (PCA). The AWCD of each plate was calculated as follows:

$$\mathrm{AWCD}=\Sigma(C-R)/31,$$

where C is the average color developed in the control cell and R is the value for an experimental cell.

Soil microbial community activity, MBC and MBN, enzymes, organic matter and total N were analyzed by ANOVA using the SPSS13.0 software package for Windows and the least significant difference was used to compare the treatments at a 0.05 probability.

2 Results

2.1 Soil microbial community activity

AWCD was used as an indicator of microbial community activity in the soil [25]. AWCD increased gradually in all treatments with increasing incubation time after the sudangrass trial in 2008 (Figure 1A). Fertilization regimes had a significant negative effect on AWCD, with AWCD values in the N addition treatments (NK, NP and NPK) being significantly lower than those in the CK and PK treatments ($P<0.05$). After the planting of ryegrass in 2009, the total AWCD in all treatments also increased gradually with increasing incubation time in the soil (Figure 1B). AWCD values in the N addition treatments (NK, NP and NPK) were also significantly lower than those in the CK and PK treatments ($P<0.05$). Total AWCD values were highest in the CK treatment and lowest in the NP treatment.

The PCA of the absorbance of the 31 carbon sources in the Biolog Eco-plates is shown in Figure 2. The results show that the NPK and NP treatments were different from the CK, PK and NK treatments after the sudangrass trial (Figure 2A), and that there were similar substrate categories between the CK and PK treatments. Exactly 36.9% and 11.2% of the total data variability is explained by the first (PC1) and second (PC2) principal components, respectively. PCA on microbial community activity after the ryegrass trial based on carbon source usage in all treatments is shown in Figure 2B. The NP treatment was different from the other treatments, and there was also similar carbon source utilization in the CK and PK treatments. The variability explained by the first and second PCA components was 26.5% and 14.2%, respectively.

2.2 MBC and MBN

Soil MBC in the sudangrass and ryegrass rotation system is shown in Figure 3A and B. Fertilizer application significantly influenced soil MBC. After the sudangrass and ryegrass trial, MBC in the NK, NP and NPK treatments in-

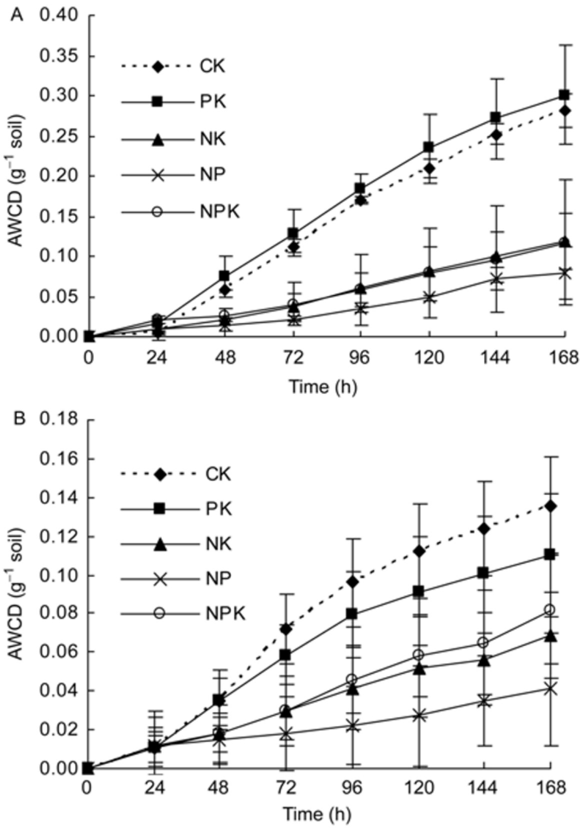

Figure 1 Soil microbial functional diversity in the sudangrass and ryegrass rotation system. A, Soil after the sudangrass trial. B, Soil after the ryegrass trial. Vertical lines indicate the standard error of all treatments.

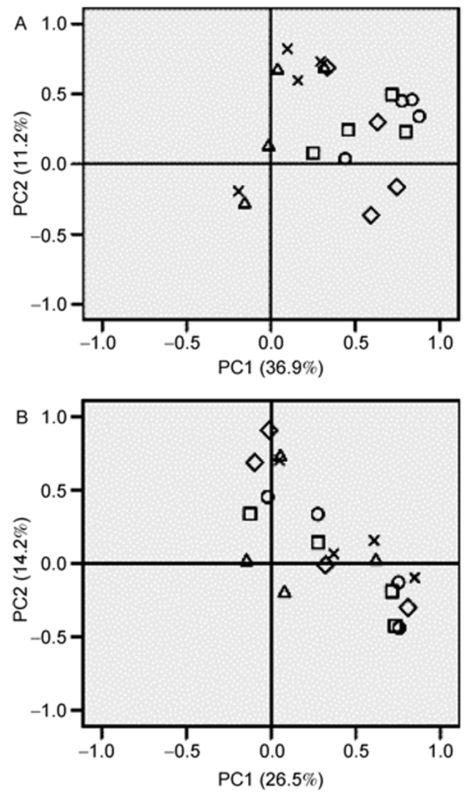

Figure 2 Principal component analysis on microbial functional diversity in all treatments. 72 h BIOLOG data adjusted for average well color development (AWCD). A, AWCD in the sudangrass trial. B, AWCD in the ryegrass trial. ○, CK; □, PK; ◇, NK; △, NP; ×, NPK.

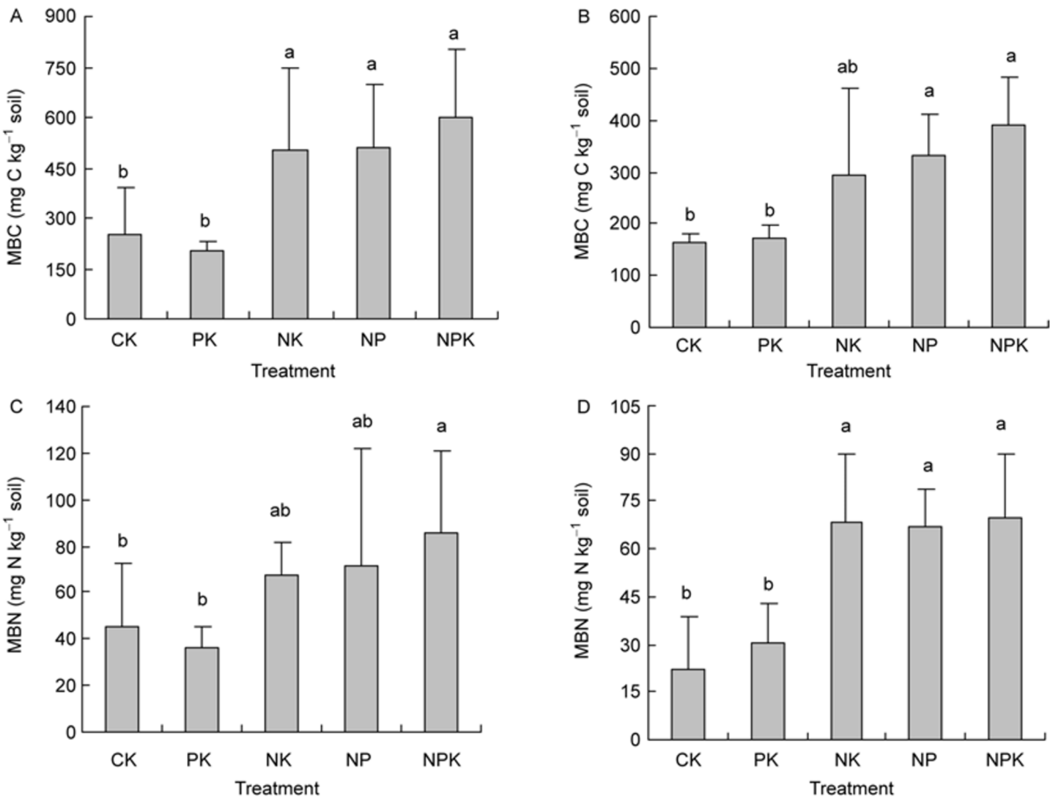

Figure 3 Soil MBC and MBN in the sudangrass and ryegrass rotation system. A, MBC in soil after the sudangrass trial. B, MBC in soil after the ryegrass trial. C, MBN in soil after the sudangrass trial. D, MBN in soil after the ryegrass trial. Small letter differences mean significant differences at $P<0.05$.

creased significantly compared with that in the CK treatment ($P<0.05$), with the NPK treatment the highest followed by the NP and NK treatments, while MBC in the PK treatment was similar to that in the CK treatment.

Fertilization regimes also influenced MBN in soil after the sudangrass and ryegrass trial (Figure 3C and D). MBN in the NPK treatment was highest after the sudangrass and ryegrass trial followed by the NP and NK treatments. MBN in the NK, NP and NPK treatments was higher than that in the CK and PK treatments, while MBN in the PK treatment was similar to that in the CK treatment.

The MBC/MBN ratios in the CK and PK treatments were 5.4–5.5 after the sudangrass trial, and were lower than those in the NP, NK and NPK treatments (7.0–7.5). After the ryegrass trial, the MBC/MBN ratio in the CK treatment was 7.3, which was higher than those in the other treatments (4.3–5.7).

2.3 Soil enzymes

Soil sucrase activities in the NP and NPK treatments were significantly higher than those in the CK, PK and NK treatments after the sudangrass trial (Figure 4A). Soil sucrase activity in the NPK treatment was highest with 63.0 mg glucose g^{-1} soil 24 h, and was lowest in the CK treatment with 25.1 mg glucose g^{-1} soil 24 h. After the ryegrass trial (Figure 4B), soil sucrase activity in the NPK treatment was highest with 34.4 mg glucose g^{-1} soil 24 h, while the PK treatment was the lowest with 24.8 mg glucose g^{-1} soil 24 h.

Soil urease activity in the NPK treatment with 125.4 μg NH_4-N g^{-1} soil 24 h was highest after the sudangrass trial (Figure 4C), although soil urease activities in all treatments were not significantly different ($P<0.05$). After the ryegrass trial (Figure 4D), soil urease activities in the NK, NP and NPK treatments were significantly higher than that in the CK treatment, while the PK treatment had the lowest soil urease activity with 53.8 μg NH_4-N g^{-1} soil 24 h.

2.4 SOM and total N

Fertilization regimes increased SOM in the sudangrass and ryegrass rotation system (Table 1). The original content of SOM was 18.50 g kg^{-1}, and after the sudangrass and ryegrass trial, SOM in the NPK treatment was highest with 25.66 g kg^{-1} and 24.01 g kg^{-1}, respectively. SOM in the CK treatment was the lowest but it also increased compared with that in the original soil.

N application increased total N in soil in the sudangrass and ryegrass rotation system (Table 1). Compared with the original soil concentration (1.05 g kg^{-1} total N), total N in the NK, NP and NPK treatments increased significantly,

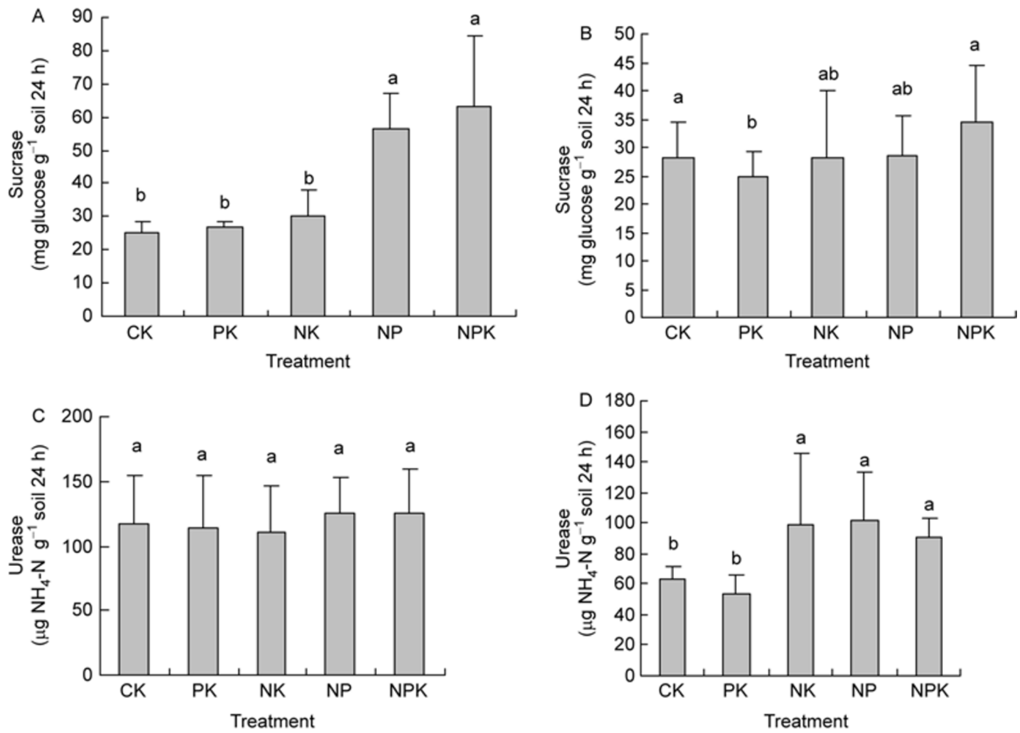

Figure 4 Soil sucrase and urease activities in the sudangrass and ryegrass rotation system. A, Sucrase in soil after the sudangrass trial. B, Sucrase in soil after the ryegrass trial. C, Urease in soil after the sudangrass trial. D, Urease in soil after the ryegrass trial. Small letter differences mean significant differences at $P<0.05$.

Table 1 Organic matter and total N in soil after the sudangrass and ryegrass trials in the rotation system[a)]

Treatment	Organic matter (g kg^{-1})		Total N (g kg^{-1})	
	Sudangrass	Ryegrass	Sudangrass	Ryegrass
CK	19.20±3.05 b	20.95±1.60 a	1.09±0.48 b	1.11±0.11 b
PK	21.33±2.36 b	21.78±3.02 a	1.07±0.19 b	1.05±0.13 b
NK	21.02±3.90 b	22.79±2.04 a	1.51±0.16 a	1.44±0.10 a
NP	22.31±2.41 ab	22.65±4.87 a	1.19±0.51 b	1.33±0.18 a
NPK	25.66±1.21 a	24.01±2.12 a	1.36±0.26 ab	1.35±0.22 a

a) Means with different letters in the same column differed significantly ($P<0.05$).

while the CK and PK treatments had total N contents similar to that in the original soil.

2.5 Correlation of MBC, MBN, sucrase, urease, SOM and total N

Correlations between MBC, MBN, sucrase, urease, SOM and total N are shown in Table 2. After the sudangrass trial, there were significant positive correlations between MBC and MBN (r=+0.989), and sucrase and urease (r=+0.882). After the ryegrass trial, there were significant positive correlations between MBC and MBN (r=+0.945), SOM and MBC (r=+0.944), SOM and MBN (r=+0.891), TN and MBN (r=+0.940), TN and urease (r=+0.958), and MBN and urease (r=+0.934). The results indicate that the soil microbial biomass and soil enzymes have an important role in the utilization of SOM and total N in sudangrass and ryegrass rotation systems.

3 Discussion

3.1 Soil microbial community activity and microbial biomass

Microbial community activity depends on many factors such as fertilizers and crop types [26,27]. C source utilization patterns reflect the catabolic potential of a microbial community [28,29]. Biolog plates have been increasingly used to characterize microbial community activity with overall color development in Biolog plates expressed as AWCD [25]. In this study, the AWCD in the N addition treatments (NK, NP and NPK) significantly decreased compared with those in the CK and PK treatments in the sudangrass and ryegrass rotation system (Figure 1). C source utilization explained by the first and second PCA components was different from the CK and PK treatments, suggesting that N application reduced the C source utilization of the soil microbial communities. Fertilizer N probably either directly or indirectly reduced microbial community activity in the soils. Similar results have been reported by Sarathchandra *et al*. [29] and Benizri *et al*. [30]. The biodiversity and activity of the soil microbial community is important to maintain soil ecosystem function [31]. Meas-

Table 2 Correlation matrices between MBC, MBN, sucrase, urease, SOM and TN in the rotation system[a)]

Variables	Sudangrass trial					Ryegrass trial				
	MBC	MBN	Sucrase	Urease	SOM	MBC	MBN	Sucrase	Urease	SOM
MBN	0.989*					0.945*				
Sucrase	0.811	0.858				0.797	0.602			
Urease	0.503	0.602	0.882*			0.855	0.934*	0.530		
SOM	0.733	0.769	0.872	0.661		0.944*	0.891*	0.767	0.697	
TN	0.770	0.702	0.271	−0.144	0.377	0.842	0.940*	0.576	0.958*	0.755

a) *, Pearson correlation is significant at the 0.05 level.

urement of the activities of microbial communities contributing to soil processes has the potential to provide particularly rapid and sensitive means of characterizing changes in soil quality [32]. Enhanced soil microbial activity was associated with high soil available N for plants [33], and soil microbial activity was likely to be greatest in row areas with a higher C input [34].

Soil microbial biomass is involved in nutrient cycling and transformation processes, which are closely related to soil fertility [35,36]. In the sudangrass and ryegrass rotation system, MBC and MBN in the NPK treatments were higher than those in the other treatments, indicating that the combination of N, P and K fertilizers could improve soil microbial biomass. Soil microbial biomass was positively associated with SOM in this rotation system (Table 2). Increased soil microbial biomass because of fertilization was a result of better crop growth and larger amounts of root residue left in the soil, which positively influenced microbial processes and development [37,38].

In this study, the most striking result observed was that the Biolog data showed significantly reduced microbial community activity after N fertilization, while MBC and MBN in the soil showed significant stimulation. Biolog plates have been used to evaluate microbial community activity, and direct sample incubation in Biolog Ecoplates may generate metabolic response patterns which were suitable for the rapid classification of heterotrophic microbial communities [4,25]. Biolog Eco-plates are tailored to ecological applications and comprise three replicate sets of 31 environmentally applicable substrates, at least nine of which are constituents of plant root exudates [39]. Although microbial community activities in our study were lower after N fertilization, maybe there were additional factors, such as other root residues, which may have affected the microbial community activities indicated in the incubation environment. Furthermore, the 31 carbon substrates assays in the Biolog Eco-plates certainly present functional diversity of microbial community, but do not reflect absolute microbial community activity which will be different from that of the soil microbial biomass. Soil microbial biomass is an important pool of plant nutrients and is highly correlated with SOM [40]. In our study, microbial biomass also increased along with increasing content of organic matter after N fertilization.

3.2 Soil enzymes

Soil enzymes have an important role in soil characteristics and are also involved in nutrient cycling processes. Enzyme activities and soil microbial biomass are closely related because transformations of the important organic elements occur through microorganisms [15,35,36]. Similar to microbial biomass in the sudangrass and ryegrass rotation system, sucrase and urease in the NPK treatments were higher than those in the other treatments, indicating that the combination of N, P and K fertilizers also improved soil enzyme activities. Some studies have shown that the application of chemical fertilizers increases soil enzyme activities [40,41], which were positively associated with SOM in this rotation system (Table 2). Soil enzymes can catalyze the transformation of SOM [35,42,43]. Increasing enzyme activities in the soil indicate improved soil fertility [40].

3.3 SOM and total N

Both SOM and total N are critical components of soil fertility and the level of organic matter and total N are influenced by fertilization regimes and crop rotation [44]. In the sudangrass and ryegrass rotation system, the level of SOM due to the combination of N, P and K fertilizers was higher than those in the other treatments. The level of SOM is considered to be a function of the net input of organic residues [45,46]. Fertilization regimes presumably affect the content of SOM by increasing aboveground and belowground production of crop residue biomass that would influence the SOM pool [17,47]. SOM in the CK treatment increased by 13.2% in the rotation system compared with that in the original soil, and it is likely that root residues added to the soil in this rotation system resulted in the increased organic C pools. Total N in soil from N application also increased by 26.7%–37.1%, while total N in the CK and PK treatments was similar to that in the original soil. However, N uptake in the CK and PK treatments occurred due to grass growth every year. Therefore, it was assumed that the crop residues and rainfall maintained the N balance in the rotation system. Similar results have already shown that SOM and total N increase with increasing N applied in continuous wheat under conventional tillage [48]. The benefits of balanced fertilization in maintaining SOM levels have been increasingly emphasized, and the increasing level of SOM due to proper fertilization regimes also means an improvement in soil fertility [42,49,50].

4 Conclusion

In a sudangrass and ryegrass rotation system, microbial community activity was changed following application of N, P and K fertilizer combinations. Soil microbial biomass, enzyme activities and soil nutrients all increased, improving soil fertility. For sustainable agricultural production in intensive cropping systems, proper fertilization regimes will sustain soil fertility for agricultural production.

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