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Responses of soil properties, microbial community and crop yields to various rates of nitrogen fertilization in a wheat–maize cropping system in north-central China

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ABSTRACT

Excessive nitrogen (N) fertilization is widespread in intensive double cropping system in China and assessment of changes in soil quality and crop production under various N application rates is important for N fertilizer management. A wheat (*Triticum aestivum* L.)–maize (*Zea mays* L.) rotation experiment was conducted from 2009 to 2012 in north-central China to study the effects of high N fertilization rates on soil chemical properties and microbial community, and to evaluate soil sustainability under reduced N inputs. The N rates tested were 0 (N0), 70 (N1), 140 (N2), 210 (N3), 280 (N4) and 350 kg N ha^{-1} (N5) in the maize season, and 0 (N0), 60 (N1), 120 (N2), 180 (N3), 240 (N4) and 300 kg N ha^{-1} (N5) in the wheat season, respectively. Soil NO_3^--N in the 0–100 cm depth, and soil electrical conductivity (EC) and nitrification potential in the 0–20 cm were significantly increased, whilst pH in the 0–20 cm was decreased with increasing N application rates. In addition, the high rates of N fertilization (N4 and N5) increased soil fungal abundance and the ratio of fungi to bacteria compared with lower N rates and the N control; however, N rates did not influence abundance of soil bacteria and actinomycetes, and total phospholipid fatty acid (PLFA). The application of N at 180 and 210 kg N ha^{-1} during the wheat and maize seasons, respectively, sustained high yields and enhanced accumulated N recovery efficiency (RE_{Nac}) compared with higher N rates. Our results indicated that the high N inputs degraded soil quality and changed microbial community structure. A 12.5–40% reduction in the farmers' conventional N application rates was practical to reduce excess N input while maintaining the sustainability of the wheat–maize cropping system in north-central China.

1. Introduction

North-central China is dominant with intensive winter wheat–summer maize cropping system, accounting for about 61% of wheat and 39% of maize production in China (Ministry of Agriculture PRC, 2012). To ensure high grain yield, farmers have been applying excessive N fertilizer (300 and 240 kg N ha^{-1} in the wheat and maize seasons, respectively) in this region over the last three decades (Cui et al., 2008; Ju et al., 2009). Since high N fertilizer inputs exceed the demand of the potential yield, significant amount of inorganic N accumulated in the subsoil and the N use efficiency (NUE) is low in this region (Ju et al., 2009). This N management practice is associated with significant N losses which have led to several negative environmental effects, such as nitrate contamination in water systems and emission of nitrous oxide to the atmosphere (Fang et al., 2006; Zhang et al., 2008; Ju et al., 2009).

High N fertilization rates generally maintain high grain yield and plant biomass; however, excessive N application could lead to soil acidification due to the massive crop uptake of base cations together with removal of economic yields, with equivalent H^+ to be released from crop to the soil, while abundant H^+ also

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generated during the oxidation of NH_4^+-N to NO_3^--N (Guo et al., 2010; Schroder et al., 2011; Liang et al., 2013). High N application also resulted in substantial soil mineral N accumulation, increased soil electrical conductivity (EC) and induced soil secondary salinization in cereal systems or greenhouse conditions (Cui et al., 2008; Shi et al., 2009; Shen et al., 2010). These changes in soil chemical properties degraded soil quality and would affect soil sustainability.

Soil microorganisms play important roles in organic matter decomposition and soil nutrient biogeochemical cycling, particularly in N transformation and cycling (Leininger et al., 2006; Cusack et al., 2011). Different microbial communities are responsible for specific ecosystem functions, and soil microbial diversity is also important in maintaining soil health and quality (Garbeva et al., 2004; Janvier et al., 2007). In addition, soil microbial community structure varies with fertilizer management practices, especially N fertilization (Chu et al., 2007; He et al., 2007; Zhong et al., 2010; Zechmeister-Boltenstern et al., 2011). It was reported that long-term application of chemical N fertilizer increased total microbial biomass and fungal abundance, but decreased bacterial abundance in a fluvo-aquic soil in Northern China (Ai et al., 2012). N fertilization did not impact total bacterial abundance, but increased the population sizes of ammonia-oxidizing bacteria and ammonia-oxidizing archaea compared with the check in a Chinese upland red soil (He et al., 2007). Other studies also found that long-term N addition to temperate ecosystems decreased microbial biomass and fungi to bacteria ratio (Frey et al., 2004; Wallenstein et al., 2006; Demoling et al., 2008). Moreover, the change in microbial community structure resulted from N addition influenced the carbon (C) and N cycling in soil ecosystem (Grandy et al., 2008, 2013; Cusack et al., 2011), and these kinds of changes are likely to negatively impact future sustainable crop production in China. However, information about the effects of continuous higher N inputs on soil microbial community is limited in the agricultural ecosystem in north-central China.

High N application was necessary to obtain high crop yield because of poor soil fertility and crop residues were removed from fields in the past. In recent decades, crop residues have been incorporated into soils (Li and Jin, 2011), this makes it necessary and possible to lower the N input in this region. To reduce excessive N fertilizer inputs and maintain soil sustainability, many N management strategies based on plant or soil nutrition diagnosis significantly decreased N fertilization rates and enhanced NUE while maintaining high grain yields (Zhao et al., 2006; Cui et al., 2008; Chuan et al., 2013), but these studies mainly focused on in-season effects of optimal N fertilizer management on crops and soils, rarely considering the sustainability of crop production. We conducted a successive seven-season winter wheat–summer maize rotation experiment in north-central China, and our objective was to examine the effects of different N application rates on (1) soil chemical properties (pH, EC and mineral N); (2) soil nitrification capacity and microbial community structure; (3) crop grain yield and N recovery efficiency (RE_N).

2. Materials and methods

2.1. Experimental site

The field experiment was conducted from 2009 to 2012 at Hengshui Dryland Farming Experimental Station (37°53′N, 115°42′E), Hebei province, north-central China. This region has a warm temperate, sub-humid continental monsoon climate. The annual mean temperature and precipitation are 12.4 °C and 550 mm, respectively, and about 70–80% of the annual precipitation occurs during the summer maize growing season. The monthly mean temperature and monthly precipitation during the experimental period are shown in Fig. S1. The soil is Calcaric Cambisols with the texture of clay loam (Ma et al., 2007), and the chemical properties of tested soil (0–20 cm) when the study was initiated were as follows: pH 8.6 (soil: water = 1:2.5), EC 2.3 dS m^{-1} (soil: water = 1:5), organic matter 15.8 g kg^{-1}, Olsen-P 20.1 mg kg^{-1}, and exchangeable K 141 mg kg^{-1}. The NH_4^+-N in the 0–20, 20–40, 40–60, 60–80 and 80–100 cm soil layers were 1.7, 4.1, 3.6, 8.4 and 10.2 mg kg^{-1}, and NO_3^--N were 36.2, 30.4, 21.8, 22.1 and 15.6 mg kg^{-1} in the corresponding soil layers, respectively.

Supplementary Fig. 1 can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2014.05.006.

2.2. Experimental design

The experiment, a typical winter wheat–summer maize rotation, started from the summer maize season of June 2009, and ended after the summer maize harvest in October 2012, covering seven successive seasonal crops in the same experimental plot. Summer maize was generally planted in middle of June after winter wheat harvest, with a row spacing of 55 cm and density of 60,000–65,000 plant ha^{-1}, and was harvested in late September or early October. Winter wheat was planted after maize harvest with a row spacing of 17 cm and seeding rate of 180–225 kg ha^{-1}, and was harvested in early or mid-June of the following year. The treatments were arranged in a randomized complete block design with three replications, and the plot size was 35 m^2 (5 m × 7 m). Six N rate treatments [60 (N1), 120 (N2), 180 (N3), 240 (N4), 300 kg N ha^{-1} (N5) and no-N control (N0)] were applied in the wheat season, and 6 N rate treatments [70 (N1), 140 (N2), 210 (N3), 280 (N4), 350 kg N ha^{-1} (N5) and no-N control (N0)] were applied in the maize season. Starter N (one half of total N amount, urea) was surface broadcast-applied by hand before sowing and incorporated into the 0–15 cm topsoil by rotary tiller, while topdressing N (the other half of total N amount, urea) was broadcast-applied at shooting stage followed by an irrigation of 60 mm water in the wheat season. In the maize season, starter N (one third of total N amount, urea) was band-applied at three-leaf stage, and topdressing N (two third of total N amount, urea) was applied at ten-leaf stage in the same way as in the wheat season. Superphosphate (90 kg P_2O_5 ha^{-1}) and potassium chloride (90 kg K_2O ha^{-1}) were used together with starter N in both crop seasons. Wheat residues were crushed and preserved in the field, and maize residues were crushed into 3–6 cm pieces and incorporated into the 0–15 cm soil by rotary tiller. The cultivars of wheat and maize were Hengguan 35 and Zhengdan 958, respectively. The amount of water irrigated each time was 60 mm. Other field management practices including pest control followed standard practices.

2.3. Crop harvest, and plant and soil sampling

At maturity, maize ears were hand harvested from an area of 7.7 m^2 (two rows, 7 m length) in the middle of each plot and shelled, air-dried grains were weighed, and moisture content was measured with a Dickey-John Tri-Grain moisture meter. Grain yield was reported at standard moisture of 15.5%. Six plants were randomly selected from each plot for a separate harvest that was used for biomass determination, and dry weights of grain and stover were determined after separation and oven-dried at 60 °C. For the wheat, three separate areas (each 2 m^2) in the middle of each plot were harvested, the dry weights of grain and straw were determined after separation and oven-dried at 60 °C, and grain yield was reported at standard moisture of 13.5%. For both crops, subsamples of grain and stover/straw were ground and analyzed for N content using the Kjeldahl method (Douglas et al., 1980).

After crop harvest, five soil cores (2 cm in diameter) were collected in each plot to a depth of 100 cm with 20 cm increments.

Table 1
Analysis of variance (p values) of soil chemical properties, nitrification potential, crop grain yield, N uptake, and accumulated N recovery efficiency (RE_{Nac}) for different N treatments and crop seasons.

Source of variation	pH	EC	NH_4^+-N	NO_3^--N	SNP	Grain yield		Crop N uptake		RE_{Nac}
						Maize	Wheat	Maize	Wheat	
Treatment (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	<0.0001	<0.0001	<0.0001	<0.0001
Season (S)	<0.0001	<0.0001	0.084	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
$T \times S$	0.355	0.388	<0.0001	<0.0001	<0.0001	0.121	<0.0001	<0.0001	<0.0001	<0.0001

EC, electrical conductivity; SNP, soil nitrification potential.

Fresh soil samples of the same soil depth per plot were mixed as a composite sample and sieved to pass a 2 mm mesh, and soil samples were immediately analyzed for mineral N (NH_4^+-N and NO_3^--N). In 2012, fresh soil samples in the 0–20 cm depth were immediately analyzed for soil nitrification potential, one part of subsamples was stored at −70 °C for microbiological phospholipid fatty acid (PLFA) analysis, and the other subsamples were air-dried for chemical analyses.

2.4. Soil chemical analyses

Soil NO_3^--N and NH_4^+-N were extracted with 0.01 mol L^{-1} $CaCl_2$ and analyzed using a flow injection analyzer (FIAstar 5000, Foss, Denmark) (Cui et al., 2008); mineral N (kg N ha^{-1}) was calculated for the 0–20, 20–40, 40–60, 60–80 and 80–100 cm depths with the bulk density values of 1.35, 1.38, 1.35, 1.36 and 1.35 g cm^{-3}. Soil water content was measured by oven drying at 105 °C. Soil EC was measured with an electrolytic conductivity meter (soil: water = 1:5) and pH with a glass electrode (soil: water = 1:2.5).

2.5. Soil nitrification potential analysis

Soil nitrification potential (soil microbial potential to nitrify added NH_4^+-N) was determined as described by Hart et al. (1994). Briefly, fresh soil samples (15 g) were placed in Erlenmeyer flasks with 100 ml mixture of 1 mM phosphate buffer (pH 7.2) and 0.75 mM $(NH_4)_2SO_4$. The slurry was shaken on an orbital shaker (180 rpm) for 24 h at 25 °C to maintain aeration in the dark. Aliquots of 10 ml were subsequently taken at 2, 6, 12, 22 and 24 h after the start of the incubation. The aliquots were centrifuged, and the supernatant was filtered and stored at −20 °C until analysis. The NO_3^--N concentrations were measured by flow injection analyzer, the nitrification potential was calculated from the slope of linear regression of nitrate concentrations over time (μg NO_3^-- N g^{-1} DW h^{-1}).

2.6. PLFA determination

Differences in microbial community structure and microbial biomass among N treatments were determined by PLFA analysis following the procedure of Wu et al. (2009). Briefly, PLFAs were extracted in a single-phase mixture of chloroform: methanol: citrate buffer (15.2 ml at a 1:2:0.8 volume ratio). The extracted fatty acids in the chloroform were fractionated into neutral lipids, glycolipids, and polar lipids using a silica-bonded phase column (SPE-Si, Supelco, Poole, UK) with chloroform, acetone and methanol, respectively. The recovered polar lipids were transesterified to the fatty acid methyl esters (FAMES) by a mild alkaline methanolysis. FAMES were quantified by gas chromatograph (N6890, Agilent) and identified with an MIDI SHERLOCKS microbial identification system (Version 4.5, MIDI, Inc., Newark, DE, USA). Nonadecanoic acid methyl ester (19:0) was added as the internal standard. PLFA concentrations were expressed in units of nmol g^{-1} soil. The abundance of individual PLFAs was indicated by their % mol abundance in each sample.

PLFAs were divided into various taxonomic groups based on previously published PLFA biomarker data (Zelles, 1997; Bossio et al., 1998; Green and Scow, 2000; Turpeinen et al., 2004). Specifically, 15:0, 16:0, 17:0, a15:0, a17:0, cy17:0, cy19:0, i14:0, i15:0, i16:0 and i17:0 were used to represent bacterial biomarkers. The polyunsaturated PLFAs 18:1ω9c and 18:2ω6,9c were chosen to indicate fungal biomarkers. The fatty acids 10Me 16:0, 10Me 17:0 and 10Me 18:0 were considered the biomarkers of actinomycetes.

2.7. Calculations

Crop N uptake was calculated based on crop N concentration and weights of grain and straw/stover.

The accumulated N recovery efficiency (RE_{Nac}) was calculated according to He et al. (2013) using the following equation:

$$RE_{Nac}(\%) = \frac{\sum_i^n U_N - \sum_i^n U_o}{\sum_i^n N_r} \times 100 \quad (1)$$

where U_N and U_0 is the total N uptake by crops (grain and stover/straw) in plots with and without N application (kg N ha^{-1}), respectively, Nr is the amount of N applied (kg N ha^{-1}), and i is the number of cultivation seasons (seven seasons in the present study).

2.8. Statistical analyses

Data were analyzed following analysis of variance using SPSS 13.0 (SPSS, Inc., Chicago, IL, USA), and the treatment means were compared using least significant difference (LSD) at the 0.05 level of probability when the main effect was significant. Student's t-test was used to illustrate the differences between two crop seasons in 2012. PLFA profiles were compared and analyzed by principal component analysis (PCA), using Minitab 16.0 (Minitab, State College, PA, USA). Stepwise multiple regression analysis (using a probability of $p < 0.05$ to accept) was applied to detect the factors influencing microbial groups.

3. Results

3.1. Soil mineral N in 0–100 cm depth

N application rate affected soil NH_4^+-N and NO_3^--N in the 0–100 cm depth (Tables 1 and 2). The variations in NH_4^+-N among N treatments were inconsistent across all seasons, and crop season did not affect NH_4^+-N. Soil NO_3^--N increased with increasing N rates in each season, and NO_3^--N in N5 treatment have highest values ranging from 307.9 to 511.6 kg N ha^{-1} during the experiment. For the N0, N1 and N2 treatments, NO_3^--N showed a gradually decreased trend with increasing of experimental time. The NO_3^--N showed an increased trend for the N4 and N5 treatments from 2009 to 2011; however, a significant decrease was observed in the 2012 maize season. For the N3 treatment, the NO_3^--N fluctuation among seasons was lower relative to other N treatments.

Table 2
Soil mineral N (NH_4^+-N and NO_3^--N) contents (kg N ha^{-1}) under different N treatments (0–100 cm).

Treatment	2009	2010		2011		2012	
	Maize	Wheat	Maize	Wheat	Maize	Wheat	Maize
NH_4^+-N							
N0	79.0 ± 3.9b	86.9 ± 6.8b	91.1 ± 8.7a	90.9 ± 2.3b	70.8 ± 4.3b	68.7 ± 1.7b	81.9 ± 1.3b
N1	93.1 ± 9.8a	101.5 ± 5.7a	97.4 ± 4.7a	102.1 ± 1.4a	71.9 ± 2.2b	80.3 ± 9.5ab	89.5 ± 4.8ab
N2	82.7 ± 4.9b	102.7 ± 9.0a	89.0 ± 15.1a	104.5 ± 4.4a	90.9 ± 6.4a	70.8 ± 7.4b	88.0 ± 4.4ab
N3	75.3 ± 3.8b	102.3 ± 4.0a	79.3 ± 3.4b	106.3 ± 6.6a	96.8 ± 9.4a	90.1 ± 2.5a	86.6 ± 3.5b
N4	74.2 ± 7.9b	76.4 ± 3.9b	88.1 ± 5.5a	104.2 ± 7.2a	95.0 ± 9.9a	77.2 ± 6.2b	94.7 ± 4.0a
N5	76.2 ± 3.2b	99.7 ± 2.8a	92.7 ± 7.9a	92.9 ± 7.9b	88.1 ± 5.8a	92.7 ± 4.3a	96.1 ± 7.8a
NO_3^--N							
N0	120.5 ± 11.6e	87.0 ± 10.3f	75.5 ± 3.7e	26.5 ± 2.3f	22.1 ± 1.6f	29.0 ± 1.9e	25.0 ± 11.0f
N1	149.4 ± 5.7d	130.4 ± 6.1e	130.2 ± 8.9d	85.0 ± 5.6e	57.2 ± 5.4e	51.5 ± 1.8e	61.4 ± 20.1e
N2	194.5 ± 11.7c	188.5 ± 7.3d	202.9 ± 3.0c	180.8 ± 6.5d	150.7 ± 3.7d	160.9 ± 9.6d	152.7 ± 14.4d
N3	188.5 ± 6.8c	225.5 ± 12.7c	233.6 ± 9.2c	249.1 ± 13.8c	217.8 ± 7.4c	213.2 ± 12.9c	215.7 ± 6.3c
N4	232.4 ± 13.6b	308.1 ± 8.8b	426.6 ± 23.5b	379.8 ± 9.7b	363.1 ± 5.9b	354.5 ± 10.5b	266.8 ± 26.7b
N5	307.9 ± 16.2a	378.8 ± 18.3a	487.1 ± 13.3a	511.6 ± 26.7a	454.7 ± 22.1a	491.5 ± 11.6a	325.4 ± 25.2a

The values are means ± standard error ($n = 3$). Different letters indicate significant differences among N treatments ($p < 0.05$) for individual crops.

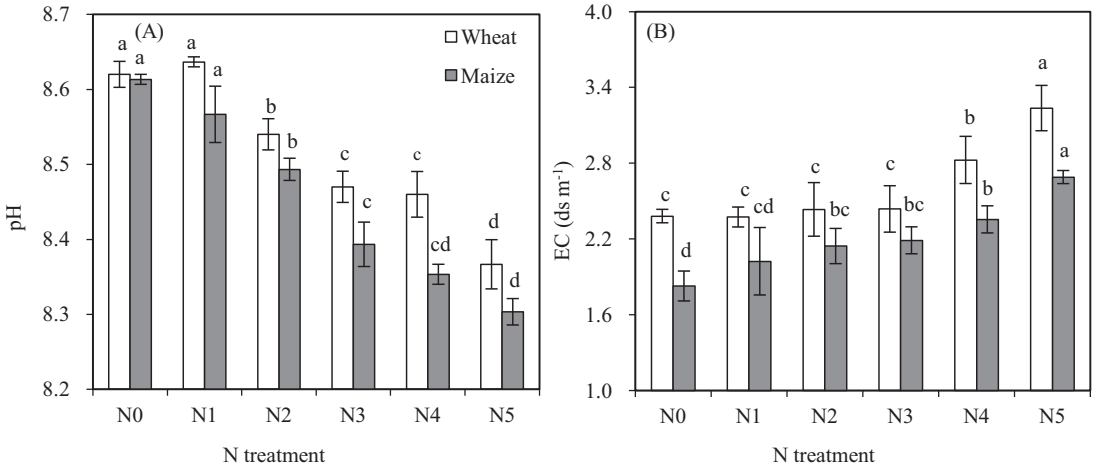

Fig. 1. Soil pH (A) and EC (B) under different N treatments in 2012 (0–20 cm). Error bars are standard error ($n = 3$). Different letters indicate significant differences among N treatments ($p < 0.05$) for individual crops.

3.2. Soil pH and EC in the 0–20 cm depth

In 2012, N application rate and crop season significantly affected soil pH and EC (Table 1). Soil pH decreased with increasing N rates in both crop seasons (Fig. 1A). By the end of the experiment, soil pH in the N5 treatment decreased by 0.31 as compared to the N0 treatment. On the contrary, soil EC increased with increasing N rates, and the N5 treatment increased soil EC by 0.85 and 0.86 dS m^{-1} compared with the N0 treatment in the wheat and maize seasons, respectively (Fig. 1B). The soil EC was higher for the N0, N4 and N5 treatments in the wheat season than in the maize season.

3.3. Soil nitrification potential

Soil nitrification potential increased with increase in N rates for both crops, and the nitrification potential was 7–9 times greater for the N5 treatment than for the N0 treatment (Fig. 2). The impacts of crop season on soil nitrification potential occurred only in the N4 and N5 treatments.

3.4. Microbial community structure based on PLFA analysis

PLFA analysis was used to determine the microbial community structure. A total of 40 PLFAs in the wheat season soil and 35 PLFAs in the maize season soil were detected and used as measures of total PLFA and the abundance of microbial groups. N application rate or crop season affected total PLFA and different microbial groups, but there were no interaction affects (Table 3). There were no differences in the abundances of total PLFA, bacterial and actinomycetes among N treatments in each crop season except that the

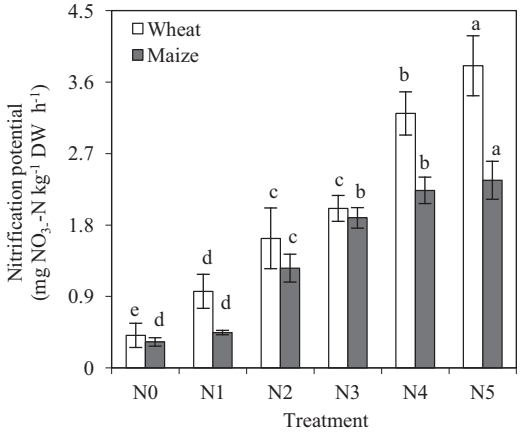

Fig. 2. Soil nitrification potential under different N treatments in 2012 (0–20 cm). Error bars are standard error ($n = 3$). Different letters indicate significant differences among N treatments ($p < 0.05$) for individual crops.

Table 3
Analysis of variance (p values) of the effects of N treatment and crop season on microbial groups.

Source of variance	Total PLFA	Bacteria	Fungi	Actinomycetes	Fungi/Bacteria
Treatment (T)	0.309	0.952	<0.0001	0.313	<0.0001
Season (S)	<0.0001	0.012	0.305	0.037	0.544
$T \times S$	0.965	0.93	0.972	0.317	0.911

actinomycetes was higher in the N1 treatment than in the N0 treatment in the maize season (Fig. 3A–C). Crop season influenced the abundances of total PLFA, bacterial and actinomycetes, but there were no consistent results in these three indexes across all N treatments. The N4 and N5 treatments enhanced fungal abundance compared with the N0–N3 treatments in the wheat season, and increased fungal abundance compared with the N0–N2 treatments in the maize season (Fig. 3D). The ratio of fungi to bacteria followed a similar trend to that observed for fungi among N treatments or seasons (Fig. 3E).

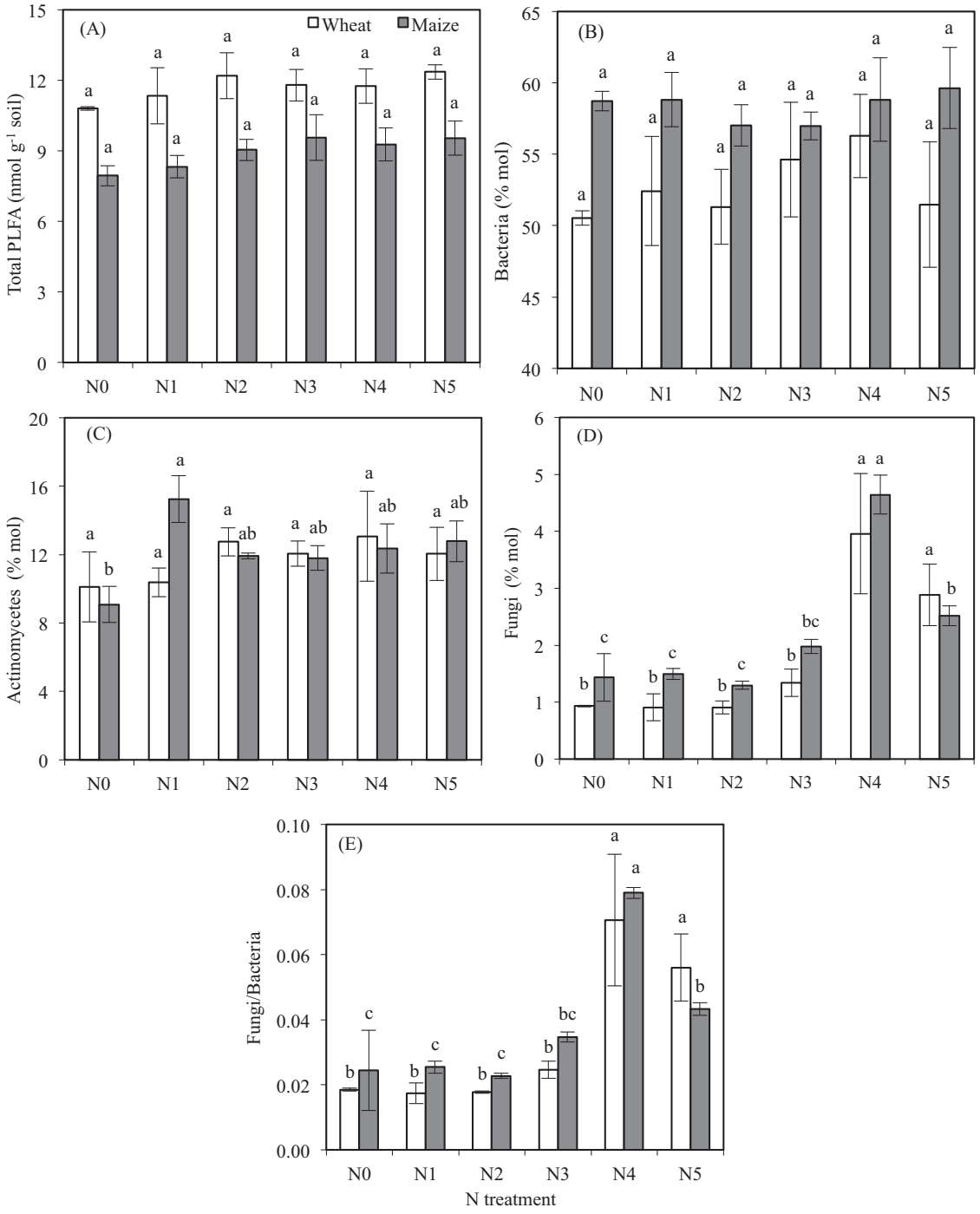

Fig. 3. Total PLFA (A), the abundances of bacteria (B), fungi (C), and actinomycetes (D), and fungi/bacteria ratio (E) under different N treatments in 2012 (0–20 cm). Error bars are standard error ($n = 3$). Different letters indicate significant differences among N treatments ($p < 0.05$) for individual crops.

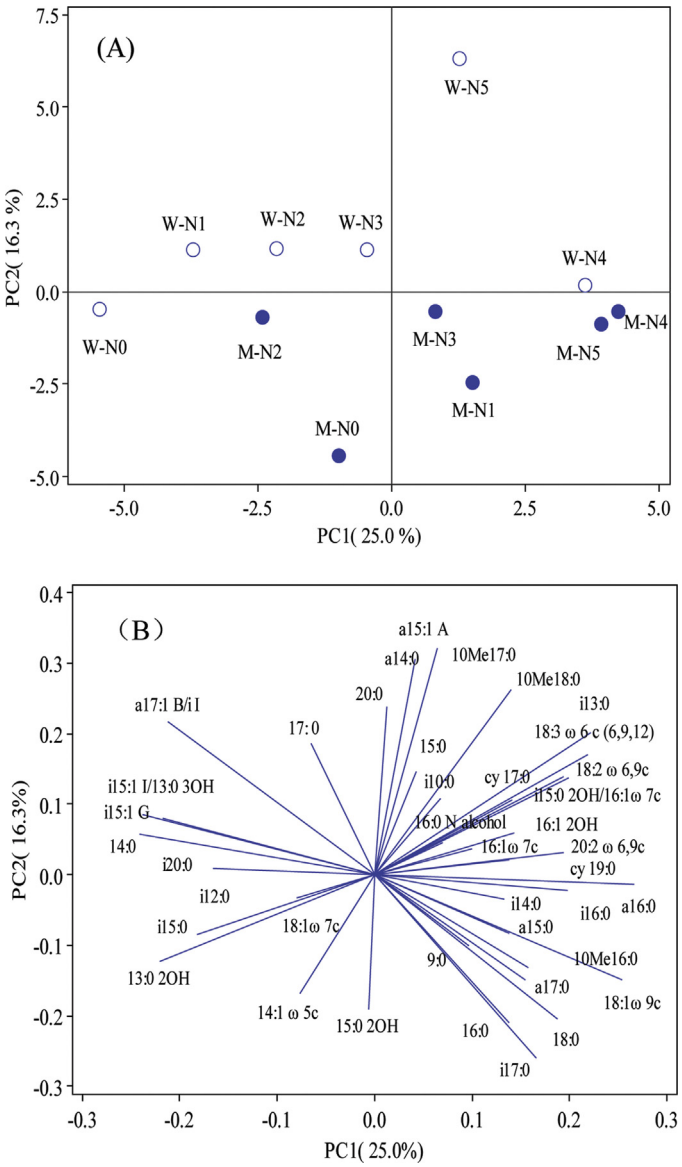

Fig. 4. Principal component analysis (PCA) of phospholipid fatty acids (PLFAs) (A) and loading values (B) for individual PLFA from the PCA of different N treatments for both winter wheat and summer maize seasons in 2012. W- and M- represent the data from the wheat and maize seasons, respectively.

The PCA was conducted with 35 PLFAs that were present either in wheat season or in maize season soils. The PC1 and PC2 explained 25.0% and 16.3% of the overall variance, respectively (Fig. 4A). N application rate and crop season significantly affected PC1 and PC2 scores for all N treatments (Table 4). PLFA profiles showed a significant separation of the N4 and N5 treatments from the N0 to N3 treatments in both crop seasons along PC1, and the score for the N0 and N1 treatments was higher in maize season than in wheat season (Fig. 4A). Along PC2, PLFA profiles for all N treatments in the maize season showed negative scores, but four of the five treatments in the wheat season showed positive scores with the exception of the negative score in the N0 treatment. PLFA profile scores for the N0, N1, and N5 treatments were higher in the wheat season than in the maize season along PC2.

Table 4
Analysis of variance (p values) of principal component (PC) scores for PLFAs among different N treatments and crop seasons.

	Treatment (T)	Season (S)	$T \times S$
PC1	<0.0001	0.012	0.003
PC2	<0.0001	<0.0001	0.0045

Table 5
Stepwise regression of microbial properties (dependents variable Y) and soil properties (independent variable X) in soil ($p < 0.05$).

Dependents (y)	Independents (x)	R^2
Total PLFA	ns	–
Bacteria	NH_4^+-N	0.378
Fungi	pH	−0.529
	Nmin	0.369
Actinomycetes	ns	–
Fungi/bacteria	Nmin	0.356

Nmin, mineral N (NH_4^+-N + NO_3^--N).
ns, no variable was detected by stepwise regression analysis to be correlated with a corresponding microbial property.

PC loadings for individual PLFAs are shown in Fig. 4B. These data and PC scores indicate that higher N application rates increased the proportion of saturated fatty acids (18:0, a16:, a17:0, i13:0, i16:0 and i17:0), polyunsaturated fatty acids [18:2ω6,9c, 18:3ω6c (6,9,12) and 20:2ω6,9c], cyclopropyl fatty acids (cy17:0 and cy19:0) and methyl branched fatty acids (10 Me16:0 and 10 Me18:0) (biomarkers of actinomycetes), and the fatty acid 18:2ω6,9c was detected only in the N4 and N5 treatments. Lower N rates enhanced the proportion of saturated fatty acids (14:0, 17:0, i10:0, i15:0 and i20:0) and monounsaturated fatty acids (i15:1G, a17:1 B/i I, 14:1ω5c and 18:1ω9c).

3.5. Stepwise multiple regression analysis

Stepwise multiple regression analyses showed that soil properties did not affect total PLFA and actinomycetes; however, bacterial abundance was significantly positively correlated with NH_4^+-N, fungal abundance was significantly negatively correlated with pH, and soil mineral N was an important factor that significantly influenced fungi and the ratio of fungi to bacteria (Table 5).

3.6. Crop grain yield, N uptake and RE_{Nac}

Analysis of variance indicated that N application rate and crop season significantly affected grain yield (for the same crop) except that no yield differences were found among N treatments in the first maize season in 2009 (Tables 1 and 6). From the second season of wheat in 2010, the N0 and N1 treatments in both 2010 and 2011 and the N0, N1 and N2 treatments in 2012 were associated with low wheat yields, and the N3, N4 and N5 treatments generally resulted in similarly higher wheat yields in the same period. For the maize, the N0 treatment decreased yield than the N3 and N4 treatments in 2010. General, the N2, N3, N4 and N5 treatments resulted in similar high maize yields from 2009 to 2012. There were no differences in total crop yield among the N3, N4 and N5 treatments, but the total crop yield was significant lower in the N2 treatment compared with that in the N4 treatment.

N application rate, crop season, and their interaction all significantly influenced crop N uptake (for the same crop) and RE_{Nac} (Table 1 and Table S1). Crop N uptake increased with increasing N rates in each crop season. The variations in wheat or maize N uptake among seasons were inconsistent for different N treatments, the higher maize N uptake in 2009 relative to other seasons likely result from higher soil residual N. There was no distinct trend in RE_{Nac} among N treatments in 2009. From 2010 to 2012, the RE_{Nac} decreased with increasing N rates in each crop season, and increased with increasing experimental seasons for the same N treatment.

Table 6
Crop grain yields (kg ha^{-1}) of summer maize and wheat under different N treatments.

Treatment	2009	2010		2011		2012		Total yield
	Maize	Wheat	Maize	Wheat	Maize	Wheat	Maize	
N0	9894 ± 239a	3692 ± 279c	7804 ± 17b	4369 ± 111d	8887 ± 165c	3426 ± 222d	8845 ± 107c	46,919 ± 159d
N1	9614 ± 518a	4766 ± 56b	8367 ± 362ab	5771 ± 47c	9336 ± 81bc	6059 ± 159c	9483 ± 61b	53,397 ± 756c
N2	9529 ± 423a	4949 ± 121ab	8463 ± 156ab	6164 ± 208ab	9628 ± 72abc	7016 ± 197b	10,085 ± 243a	55,834 ± 925b
N3	9963 ± 399a	5246 ± 134ab	8680 ± 193a	6274 ± 83a	10,026 ± 389ab	7597 ± 123a	10,249 ± 168a	58,016 ± 427ab
N4	9488 ± 476a	5506 ± 297a	8971 ± 331a	6566 ± 183a	10,251 ± 315a	7742 ± 59a	10,412 ± 237a	58,942 ± 1330a
N5	9352 ± 156a	5537 ± 160a	8054 ± 58ab	6367 ± 152a	10,046 ± 378ab	7633 ± 83a	9930 ± 48a	56,921 ± 332ab

Total yield, total grain yield of maize and wheat in seven crop seasons.
The values are means ± standard error ($n = 3$). Different letters indicate significant differences among N treatments ($p < 0.05$) for individual crops.

Supplementary Table 1 can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2014.05.006.

4. Discussion

4.1. Soil chemical properties

In dryland soils, NH_4^+-N from chemical N fertilizers could be quickly oxidized to NO_3^--N, and different N fertilization rates and crop stages had no effects on NH_4^+-N but NO_3^--N increased with increasing N rates as the main form of mineral N (Ju et al., 2003; Wu et al., 2008). Our results showed that crop season did not affect soil NH_4^+-N in the 0–100 cm depth, but residual NH_4^+-N was slightly higher compared with that reported by Ju et al. (2003) in north-central China and by Yang et al. (2013) in the Loess Plateau of China, and a possible explanation for this discrepancy could be related to high NH_4^+ sorption in clay soil texture in this study which decreased the nitrification of NH_4^+-N (Dempster et al., 2012). However, NO_3^--N significantly increased with increasing N application rates. While, in despite of crop residues retention, the NO_3^--N in the low N rate treatments (N1 and N2) showed a decreased trend across the whole experimental period because the crop N depletions were higher than the N inputs, and the decline would be continued with increasing experimental times. However, the NO_3^--N in the high N rate treatments (N4 and N5) showed an increased trend from 2009 to 2011 due to the N inputs significantly exceed the crop N uptakes, and a significant decrease in 2012 maize season possible due to high precipitation which leached excess NO_3^--N below the 100 cm. The NO_3^--N had no great change in the N3 rate treatment from 2009 to 2012 because of better balance between N inputs and crop N uptakes. These results are consistent with previous studies in this region (Fang et al., 2006; Ju et al., 2003) and in the Loess Plateau of China (Gao et al., 2009), indicating that excessive N fertilization resulted in high soil residual NO_3^--N. The high subsoil residual NO_3^--N could be easily leached into ground water, and causes severe environment problems (Beaudoin et al., 2005; Ju et al., 2009). It is uncontentious that N fertilization lower soil pH (Blake et al., 1999; Guo et al., 2010; Schroder et al., 2011; Liang et al., 2013). Schroder et al. (2011) found that 32-year N fertilization resulted in a pH decline of 0.3–1.1 compared to the CK, which was associated with a decrease of wheat yield in the Great Plain, US. Guo et al. (2010) also reported that the use of N fertilizer decreased pH by 0.34 and 0.55 under cereals and cash crops in major China croplands from the 1980 to 2000s, respectively. Similarly, we also found that the N fertilization decreased soil pH, and the high N rate (N5 rate) decreased pH by 0.31 relative to the N control (N0) after seven successive seasons of cropping. Compared with the results above, the decline rate of pH was greater in high N fertilization treatment after seven-season cropping in our study, indicating that high N inputs intensified soil acidification.

Soil EC has traditionally been used to indicate the magnitude of salts in soil that are harmful to plant growth. The increased soil EC observed in high N rate treatments relative to the non-N control suggested that higher N inputs induced soil secondary salinization. Other researches also demonstrated that high N inputs enhanced EC in soils from greenhouse vegetable production in Eastern China (Shen et al., 2010). From the above, it was indicated that high rates of N fertilization resulted in greater soil NO_3^--N accumulation, induced soil acidification and secondary salinization. These changes in soil properties would degrade soils and environmental quality in this region, furthermore, these degradations might be expected to accelerate under high N fertilization in the future. Therefore, great attention should be paid in high N application area and optimized N management strategies should be taken to sustain soil quality and productivity in this highly intensified region.

4.2. Soil nitrification potential and microbial community structure

Successive high N inputs (N4 and N5 rates) significantly increased soil nitrification potential compared with the treatments with lower N rates and the control. Studies also found that long-term application of chemical N fertilizer increased soil nitrification potential in an alkaline soil in Northern China, because the decrease of soil pH increased the abundance of ammonia-oxidizing bacteria (Chu et al., 2007; Shen et al., 2008). In addition, higher nitrification potential also explained higher soil NO_3^--N accumulation in the high N rate treatments. However, the N fertilization decreased soil nitrification potential in spite of a significant reduction in pH in a Chinese upland red soil (He et al., 2007). This inconsistency may be due to the different soil types and pH ranges (Girvan et al., 2003; He et al., 2007).

N fertilization rate did not influence abundance of soil bacteria although higher soil nitrification potential may result from increased ammonia-oxidizing bacteria, which is only a small fraction of the total bacterial population (He et al., 2007). N fertilization did not alter total PLFA and actinomycetes abundance as well, but high N application (N4 and N5) increased fungal abundance and the ratio of fungi to bacteria. Zhong et al. (2010) indicated that soil bacteria were sensitive to organic manure application and soil fungi were sensitive to mineral fertilizer; however, actinomycetes were not affected by the fertilization. Stepwise regression analysis showed that soil pH and N nutrient were the key factors affecting the microbial properties, and the pH was more correlated with fungi abundance than soil nutrients. Because soil fungi prefer an acid soil environment (Gong et al., 2009; Rousk et al., 2011), the lower soil pH resulted in a high proportion of fungi and increased the ratio of fungi to bacteria for high N rate treatments in the current study. Analyses from PCA displayed that crop seasons also influenced soil microbial community structure, because different crop residual qualities and soil temperatures regulated growth and population of specific microbial communities (Cusack et al., 2011; Tiemann and Billings, 2011), but the effects of crop season on soil microbial community was less than the higher N

fertilization. Our data demonstrated that high N application changed the soil microbial community structure through affecting soil chemical properties in spite of the fact that N rates did not influence total PLFA. These changes in soil microbial community structure may alter soil ecosystem function, such as organic matter decomposition and soil nitrification capacity, and then affect soil C and N cycles in agricultural ecosystem.

4.3. Crop grain yield and N recovery efficiency

N application had no effect on grain yield in the first maize season in 2009, indicating that residual soil available N meet or exceed the crop N demand for high yield (the mineral N in the 0–100 cm at the beginning of this experiment was 428 kg N ha^{-1}) and a reduction in N fertilizer inputs was necessary and possible in this region. The N2 rate sustained similar high maize yield to the N3, N4 and N5 rates treatments during this experiment, however, the gradual decrease in soil mineral N resulted from crop N depletion would limit high crop yield with the increase in experimental time. Compared with the high N rates (N4 and N5), the N3 rate (180 and 210 kg N ha^{-1} in the wheat and maize seasons, respectively) decreased subsoil NO_3^--N accumulation, increased NUE, and sustained high total crop yield during the experimental seasons. The crop residues N retention played a great role in sustaining sufficient soil N supply (Ju and Christie, 2011). The optimal N rates applied here were 40% and 12.5% lower in the wheat and maize seasons, respectively, compared with local farmers' practices (300 and 240 kg N ha^{-1} in the wheat and maize seasons, respectively), and was practical to ensure sustainable soil quality and crop productivity in this successive seven-season rotation experiment.

5. Conclusions

Our study indicated that high N fertilization rates increased NO_3^--N accumulation in the subsoil, caused soil acidification and secondary salinization, and altered microbial community structure and increased soil nitrification capacity. Compared with high N fertilization, the application of N fertilizer at 180 and 210 kg ha^{-1} in the wheat and maize seasons, respectively, reduced soil residual NO_3^--N, enhanced NUE, and alleviated the degradation in soil quality, while sustaining high crop yields. Therefore, by preserving crop residues in field, the optimal N application rates are 12.5–40% lower than the local farmers' practice, which are practical to maintain the sustainable soil quality and productivity in the intensive wheat–maize cropping system in north-central China.

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