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RESEARCH ARTICLE

Dynamic changes in soil chemical properties and microbial community structure in response to different nitrogen fertilizers in an acidified celery soil

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ABSTRACT

To determine the effects of different kinds of nitrogen fertilizer, especially high-efficiency slow-release fertilizers, on soil pH, nitrogen (N) and microbial community structures in an acidic celery soil, four treatments (CK, no N fertilizer; NR, urea; PE, calcium cyanamide fertilizer; and SK, controlled-release N fertilizer) were applied, and soil pH, total soil N, inorganic N, and soil microbial biomass C were analyzed. Phospholipid fatty acids (PLFAs) were extracted and detected using the MIDI Sherlock microbial identification system. The PE treatment significantly improved soil pH, from 4.80 to >6.00, during the whole growth period of the celery, and resulted in the highest celery yield among the four treatments. After 14 d application of calcium cyanamide, the soil nitrate content significantly decreased, but the ammonium content significantly increased. The PE treatment also significantly increased soil microbial biomass C during the whole celery growth period. Canonical variate analysis of the PLFA data indicated that the soil microbial community structure in the CK treatment was significantly different from those in the N applied treatments after 49 d fertilization. However, there was a significant difference ($P < 0.05$) in soil microbial community structure between the PE treatment and the other three treatments at the end of the experiment. Calcium cyanamide is a good choice for farmers to use on acidic celery land because it supplies sufficient N, and increases soil pH, microbial biomass and the yield of celery.

1 Introduction

In the past 30 years, a large of chemical fertilizers and other resources have been used in agriculture (Guo et al., 2010). Some greenhouse vegetable systems even receive N fertilizer

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at rates of >4000 kg·ha^{-1}·year^{-1} (Ju et al., 2007). High levels of fertilizer inputs cause serious soil acidification problems, with pH decreases of 0.3 to 0.8 units in greenhouse vegetable systems in China (Guo et al., 2010; Liang et al., 2013; Cai et al., 2015). The production of protons via the nitrification process after urea and ammonium nitrogen fertilization is considered the major driver of soil acidification. Moreover, ammonium can displace base cations (Ca^{2+} , Mg^{2+} , K^+, Na^+) binding to the soil surface and make them enter the soil solution as accompanying ions with the leaching nitrate (Matschonat and Matzner, 1996), reducing their ability to buffer against acidification (Lucas et al., 2011). Soil acidification can lead to deficiencies in phosphorus, potassium, magnesium, and calcium, and the accumulation of aluminum and manganese in soil (Ruan et al., 2012; Tian and Niu, 2015). Moreover, nitrogen fertilizers application suppresses both soil microbial biomass and microbial diversity (Zhou et al., 2017). Recently, a meta-analysis revealed that N addition decreased the relative abundance of Actinobacteria and Nitrospirae in soil (Wang et al., 2018). This degradation of soil quality reduces productivity and profitability for farmers (Wells et al., 2000). This problem has become a major concern for scientists, agricultural policymakers and farmers (Liang et al., 2013). The Ministry of Agriculture of the People's Republic of China formulated plans to obtain a target of "zero growth" in the amount of chemical fertilizers used on crops by 2020. Many scientists have designed knowledge-based optimum fertilization management practices to increase the efficiency of use of fertilizers (Ju et al., 2007; Yang et al., 2016). Liming (Zhang et al., 2015), manure application (Noble et al., 1996; Cai et al., 2015), and slow-release fertilizer use (Gu et al., 2014) all increase soil pH. Various fertilization modes have been applied during crop cultivation. However, most of the new technologies available to farmers were obtained from fertilizer sellers, and their effects on soil quality were seldom considered.

Celery is a high-value, low-acreage crop that receives high rates of N fertilizer to maximize yield (El-Sayed et al., 2011). In China, celery is one of the most important vegetables, produced on about 550 000 ha with a farm gate value of about US$10 billion. Many studies have shown that different N fertilizers have different effects on soil chemical and biological properties (Marschner et al., 2003; Van der Bom et al., 2018). In this study, three nitrogen fertilization treatments, including urea, calcium cyanamide and a commercial "celery special fertilizer" were compared to determine their effects on soil properties and microbial community structure. The objectives of this study were to select the optimal fertilizer treatment and to understand the underlying microbial mechanisms.

2 Materials and methods

2.1 Study site

Field work was conducted in Sanqin village, Jiangbei District, Ningbo, China (29°57′57″N, 121°22′59″E). Farmers have grown rice there for >20 years, but the site changed to greenhouse vegetable land in 2012. The total annual precipitation averages 1440 mm and the annual mean air temperature is 16.1°C. The field experiment site has an area of 450 m^2. Soil chemical and physical properties were: pH 4.80 (1:2.5 w/v, soil/water), total carbon 28.85 g·kg^{-1} soil, total nitrogen 3.56 g·kg^{-1} soil, nitrate (NO_3^-) 14.8 mg·kg^{-1}, ammonium (NH_4^+) 52.4 mg·kg^{-1}, available P 219.0 mg·kg^{-1} and available K 392.9 mg·kg^{-1}.

2.2 Experimental design

Twelve study plots were established in a random block design with three replicates. Each plot had an area of 37.5 m^2 (length 25 m, width 1.5 m). The field experiment was conducted from 23 December 2013 to 10 March 2014. Celery (*Apium graveolens* Linn.) was cultivated in each plot with a number of 3000 plants. Four treatments were applied: (1) no N fertilizer (CK); (2) urea (NR); (3) calcium cyanamide (PE); (4) celery special controlled-release fertilizer (SK). Based on the recommended fertilization regime, before planting, all the plots were applied the same amounts of nitrogen, phosphate and potassium fertilizers, with application rates 100 kg N·ha^{-1}, 90 kg P_2O_5·ha^{-1} and 90 kg K_2O·ha^{-1}, respectively. The dates of celery transplanting and harvesting were 11 January 2014 and 10 March 2014.

2.3 Analytical methods

Soils were sampled on 23 December 2013 (0 d), 6 January 2014 (14 d), 10 February 2014 (49 d) and 10 March 2014 (78 d), respectively. The soil was sieved through a 2.0 mm mesh, and separated into three parts. The first part was air-dried for pH, available P, available K, total C and N analysis. The second part was stored at 4°C for microbial biomass C and N analysis, except that inorganic N was detected immediately. The third part was freeze-dried for PLFA extraction.

Soil pH in water was detected at a ratio of 1:2.5 (w/v) using a pH meter (PE20, Mettler Toledo, Switzerland). Total C and N were determined using a CNS Element Analyzer (Vario MAX C/N; Elementar, Germany). Soil available P was extracted with 0.5 M $NaHCO_3$ (pH 8.5) (soil: $NaHCO_3$ = 1:20), then shaken on a horizontal shaker for 30 min, and determined by colorimetry (Bao, 1999). Exchangeable K was extracted with 1 M CH_3COONH_4 (pH 7.0) (soil: $NaHCO_3$ = 1:10) and shaken for 30 min, and measured by atomic absorption spectrometry (Bao, 1999). Soil NH_4^+ and NO_3^- were extracted with 1 M KCl (soil: KCl = 1:10) and shaken for 1 h, and detected colorimetrically using a micro-plate reader (Spectramax M5, Molecular Devices, USA) (Shand et al., 2008).

Soil microbial biomass C (MBC) analyses was performed using the chloroform fumigation extraction method (Brookes et al., 1985; Wu et al., 1990), and a K_{EC} factor of 0.45 was used to calculate MBC. PLFAs were analyzed according to the method of Li et al. (2016). Approximately 2.0 g of freeze-dried soil was extracted with a total of 22.8 mL of

methanol-chloroform-citrate buffer mixture (2:1:0.8 v/v/v, 0.15 M, pH 4.0). A silic acid column was used to separate the phospholipids from neutral- and glycol-lipids. The phospholipids were methylated by a mild-alkali methanolysis. PLFA methyl esters were identified and quantified by gas chromatography (Column HP 5, 50 m length, 0.2 mm i.d, 0.33 μm film thickness; Agilent Technologies Inc., Santa Clara, USA). The detailed equipment information and running conditions were as described by Thornton et al. (2011). The fatty acid 19:0 was added prior to methylation as an internal standard.

2.4 Statistical analysis

All data are the means of three replicates. One-way analysis of variance was performed to test the results, and significant differences ($P < 0.05$) between means was calculated by *t* test. The PLFA data were expressed in molar percent (mol%) for multivariate analyses. After first reducing the dimensionality by principal component analysis (PCA), the absorbance data were assessed by canonical variate analysis (CVA). Multivaritate analyses were conducted using GenStat 16th Edition (VSN International, Oxford, UK), and all the other analyses were performed using DPS v7.05.

3 Results

3.1 Changes in soil chemical properties

The NR and SK treatments had no significant effect on celery yield. Application of calcium cyanamide (PE) resulted in the highest yield (37.5 $t \cdot ha^{-1}$), which was nearly 1.5 times higher than that in the control (CK) (Fig. 1). Soil pH significantly increased from 4.80 to 6.53 at 14 d after application of calcium cyanamide (Fig. 2A). The increasing trend lasted until the end of the experiment. At 78 d after application, soil pH was 6.16 in the PE treatment, which was much higher than those in the other treatments (Fig. 2A). There was no significant difference in soil pH among the CK, NR and SK treatments during the whole growth period (Fig. 2A). Soil available P and K significantly increased in the PE treatment at 14 d after application of calcium cyanamide (Fig. 2B and Fig. 2C). The content of available P was apparently higher in the CK and PE treatments than that in the SK treatment on 49 d (Fig. 2B). On 78 d, soil available P contents were not significantly different among the four treatments (Fig. 2B). The available K content showed no significant difference between the four treatments both on 49 d and 78 d (Fig. 2C).

The total soil N content was lowest in the SK treatment during the experiment (Fig. 2D). There was no significant difference in soil total N in the CK, NR and PE treatments (Fig. 2D). From 14 d after application of calcium cyanamide, the soil NO_3^- content significantly decreased in the PE treatment (Fig. 2E). There was no significant difference in NO_3^- content between the treatments after 49 d fertilizer application (Fig. 2E). On 78 d, the concentration of NO_3^- in the SK and NR treatments was much higher than those in the PE and CK

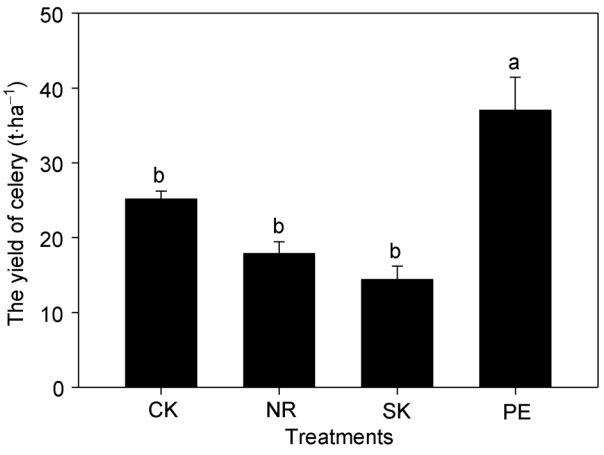

Fig. 1 Effect of different N fertilizers on celery yield. CK, control; NR, urea; PE, calcium cyanamide fertilizer; SK, celery special fertilizer.

treatments (Fig. 2E). Calcium cyanamide fertilization significantly improved the content of NH_4^+ in soil 14 d from application (Fig. 2F). On 49 d, there was no significant difference in NH_4^+ content between the treatments (Fig. 2F). The contents of soil NH_4^+ in the SK and PE treatments were significantly higher than those in the NR and CK treatments at the end of experiment (Fig. 2F).

3.2 Changes in soil microbial biomass and community structure

The PE treatment significantly increased soil microbial biomass C from 414 μg $C \cdot g^{-1}$ soil to 633 μg $C \cdot g^{-1}$ soil after 14 d fertilization (Fig. 3A). On 49 d, soil microbial biomass C was significantly higher in the PE, NR and CK treatments than in the SK treatment (Fig. 3A). On 78 d, soil microbial biomass C was the highest in the PE treatment with a content of 794 μg $C \cdot g^{-1}$ soil, followed by the CK treatment (669 μg $C \cdot g^{-1}$ soil) and NR treatment (652 μg $C \cdot g^{-1}$ soil) (Fig. 3A). Soil microbial biomass C was lowest in the SK treatment (481μg $C \cdot g^{-1}$ soil) (Fig. 3A). Although soil microbial biomass C and total PLFAs were significantly correlated ($P < 0.05$) (Fig. 4), soil microbial biomass calculated by total PLFAs showed no significant differences between the four treatments (Fig. 3B).

The relative abundances of characteristic PLFAs for Gram-negative bacteria, Gram-positive bacteria, Fungal and Actinomycetes were 19.8%, 27.7%, 2.1% and 11.0%, respectively. There was no significant difference among the four treatments and the two sampling times (49 d and 78 d) (Fig. S1). Canonical variate analysis (CVA) of the PLFA data indicated that the soil microbial community structure in the CK treatment was significantly different from those in the SK, NR and PE treatments after 49 d fertilization (Fig. 5A). However when the celery was harvested (78 d), there was a significant difference ($P < 0.05$) in soil microbial community structure between the PE treatment and the other three treatments (Fig. 5B). The relative concentration of PLFAs 14:0, i14:0,

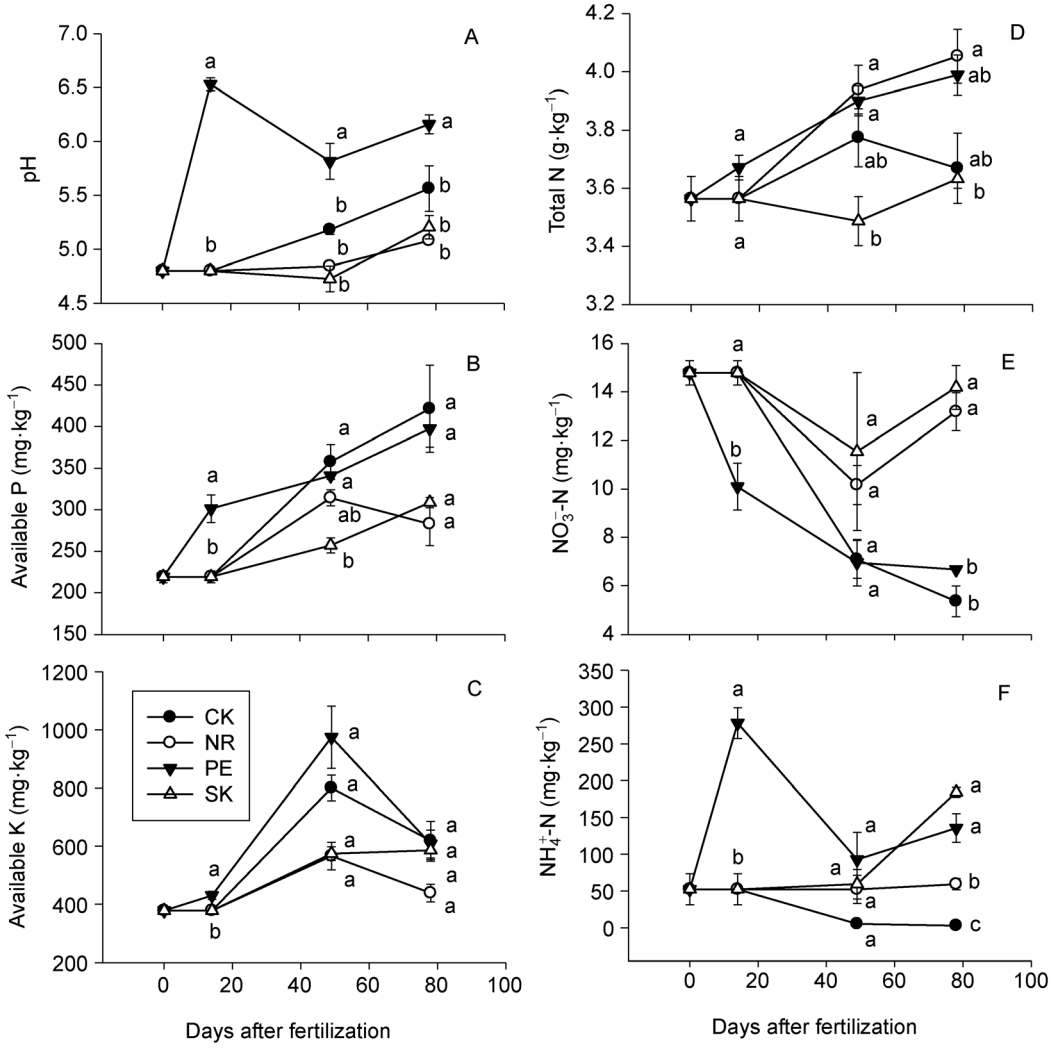

Fig. 2 Temporal variations in (A) pH, (B) available P, (C) available K, (D) total N, (E) NO_3^-, and (F) NH_4^+ in soils over the course of the field experiment. CK, control; NR, urea; PE, calcium cyanamide fertilizer; SK, celery special fertilizer. Bars are standard errors. Different letters (a, b and c) indicate significant differences between different treatments at $P < 0.05$.

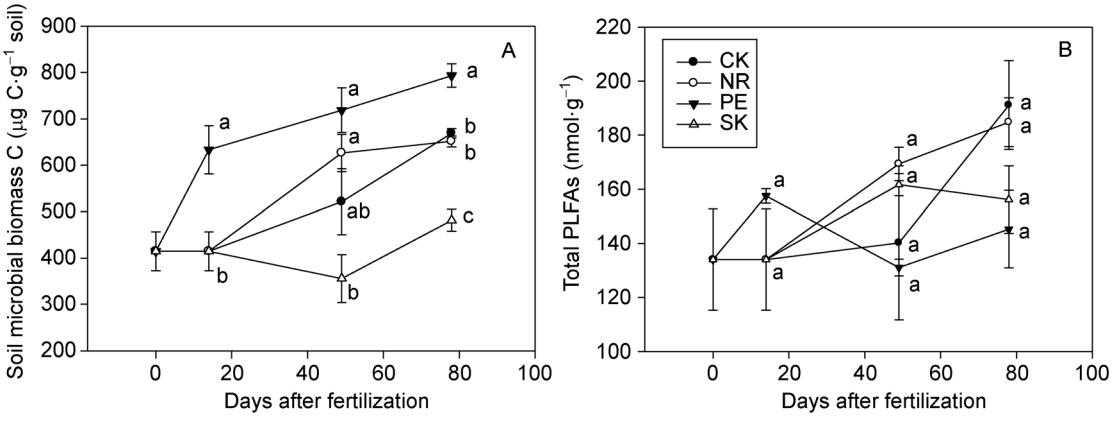

Fig. 3 The variation of soil microbial biomass C (A) and total phospholipid fatty acids (PLFAs) (B). CK, control; NR, urea; PE, calcium cyanamide fertilizer; SK, celery special fertilizer. Bars are standard errors. Different letters (a, b and c) indicate significant differences between different treatments at $P < 0.05$.

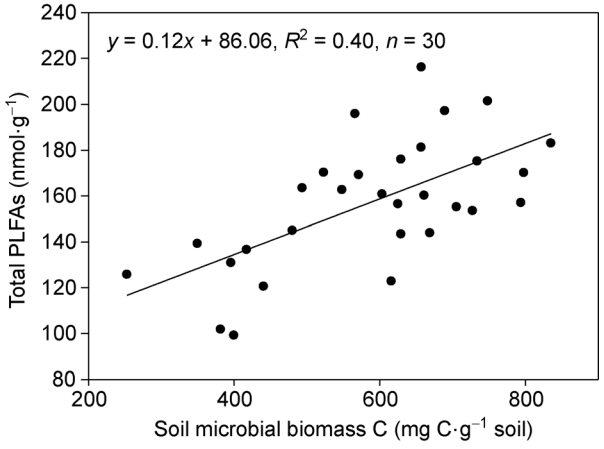

Fig. 4 Biomass C and total PLFAs in soils (R^2 = 0.40, n = 30).

br16:0, 16:1ω 11, 16:1ω 7t, 18:1ω 7, 18:2ω 8,12 and 18:2ω 6,9 was higher in the CK treatment than in the other treatments from 49 d after fertilization (Fig. 6A). After 78 d fertilization, the PE treatment had higher concentrations (mol %) of PLFAs 15:0, 18:0, i15:0, br17:0, 10Me16:0, 10Me17:0 and 10Me18:0, and lower concentrations of PLFAs a15:0, cy17:0, 16:1ω7c, 16:1ω5c, 18:1ω7, 18:1ω10, and 18:2ω6,9 compared with the other treatments (Fig. 6B). Analysis of the loading of PLFAs showed that 19:0cy was enriched in the PE treatment, but it had a lower relative abundance of 18:1ω7 and 16:1ω7c.

4 Discussion

Calcium cyanamide was one of the first synthetic nitrogenous fertilizers (Crowther and Richardson, 1932). On application to soil, it can be transformed to hydrogen cyanamide, calcium hydroxide and dicyandiamide, hence it has also been widely used as a disinfectant, herbicide, acid modified agent and a slow-release fertilizer (Oh et al., 2006). In our study, the application of calcium cyanamide significantly increased soil pH by >1 unit during the full cultivation period. Oh et al. (2006) also found that calcium cyanamide was effective in stopping soil acidification in tea fields (with an initial pH of 4.5) when applied at almost the same rate as in our study. In traditional liming treatments in China, a large amount of burnt limestone powder (49.5% CaO, usually applied at greater than 2000 $kg \cdot ha^{-1}$) is recommended to obtain increased soil pH (Li et al., 2014). The burnt limestone powder in China is usually not granulated, combined with often humid weather, may result in a proportion of the calcium oxide (CaO) reacting with water (H_2O) and carbon dioxide (CO_2) to produce calcium bicarbonate ($CaHCO_3$). This may explain the high application rates of burnt limestone used. Calcium cyanamide would be a better choice to increase soil pH compared with limestone powder.

The content of NH_4^+ in soil significantly increased after calcium cyanamide application in this study, which was consistent with Arora et al. (1987) and Xiao et al. (2018). They found that most of the inorganic N accumulated in soil after application of calcium cyanamide was present as NH_4^+, a breakdown product of urea. In soil, calcium cyanamide is hydrolyzed to cyanamide (H_2CN_2) and slaked lime ($Ca(OH)_2$), and then H_2CN_2 is converted into urea and dicyanadiamide ($C_2H_4N_4$). Both H_2CN_2 and $C_2H_4N_4$ are well known inhibitors of microbial nitrification (Mukerji, 1932; Amberger and Vilsmeier, 1979; Yamamoto et al., 2014). The addition of calcium cyanamide at a rate of 20 mg $N \cdot kg^{-1}$ soil can thoroughly inhibit nitrification activity in soils for at least two months (Arora et al., 1987).

The celery in the SK treatment had the lowest yield, around one-third of that with PE treatment. From 49 d to 78 d cultivation, total N, NO_3^- and NH_4^+ in the SK treatment increased significantly. The chemical N applied in the SK treatment was a type of polymer coated fertilizer. The release of nitrogen from the coated fertilizer to the soil might not meet

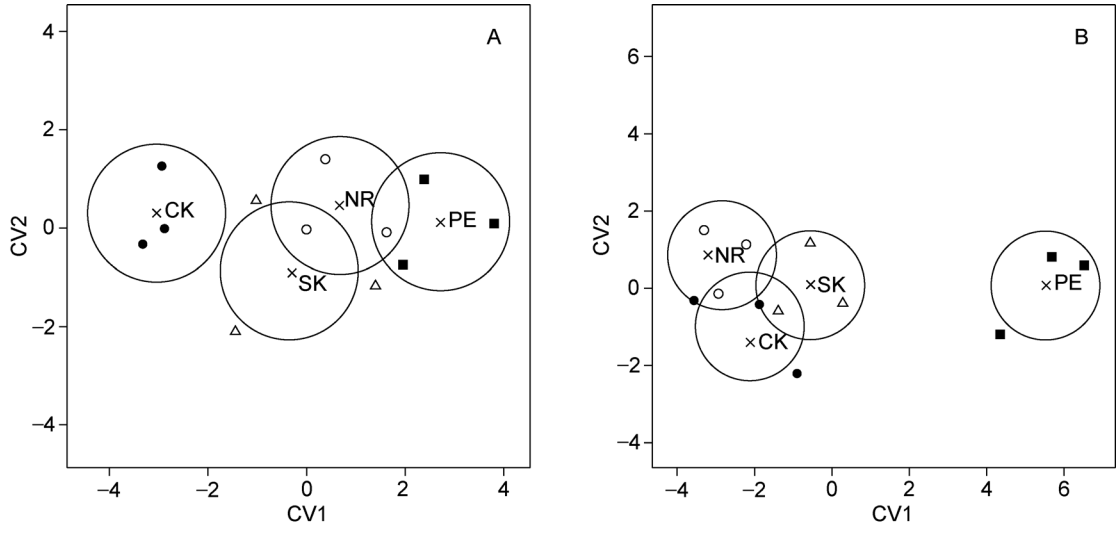

Fig. 5 Plot of ordination of canonical variates (CV). CV1 against CV2 generated by canonical variate analysis of PLFAs after 49 d (A) and 78 d (B) fertilization. CK, control; NR, urea; PE, calcium cyanamide fertilizer; SK, celery special fertilizer.

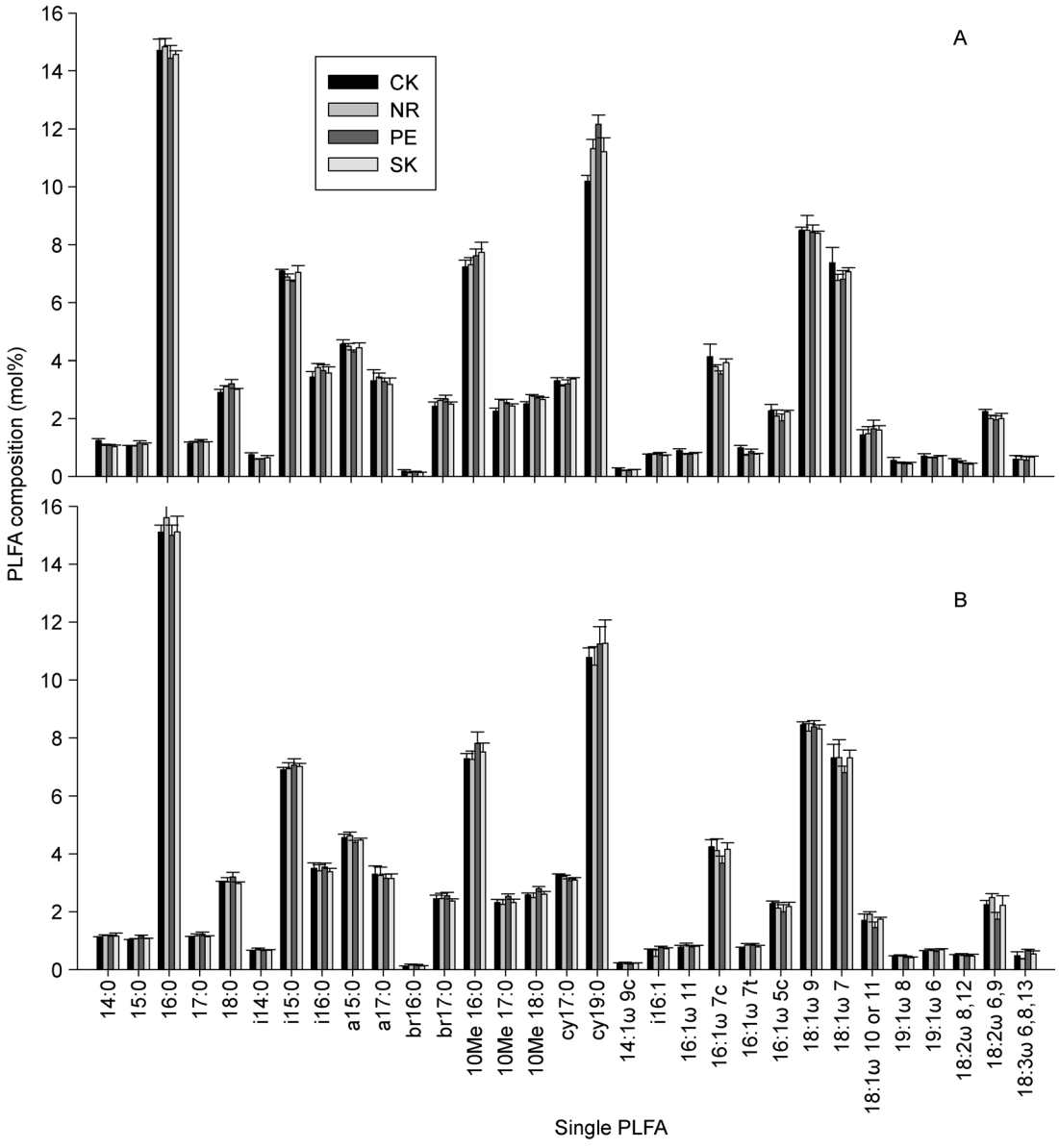

Fig. 6 Relative abundance (mol%) of PLFAs in four treatments after 49 d (A) and 78 d (B) fertilization. CK, control; NR, urea; PE, calcium cyanamide fertilizer; SK, celery special fertilizer.

the growth needs of celery, thus resulting in the low yield (Xiong et al., 2010). Although the fertilizer used in the SK treatment was called celery special fertilizer by the distributor (Agrium Advanced Technologies Inc., Canada), it was not a good choice for optimum celery yield, based on our experiment.

Calcium cyanamide can increase soil pH and release nitrogen, both of which can promote the growth of soil microbes (Zhang et al., 2008; Tian et al., 2009). Xiao et al. (2018) showed that calcium cyanamide could stimulate the population size of soil bacteria, but decrease the population size of fungi. The hydrolysates H_2CN_2 and $C_2H_4N_4$ are toxic to soil microbes (Amberger and Vilsmeier, 1979), but both of them degrade rapidly in soil. Shi et al. (2009) found that the suppressive effect of calcium cyanamide on the soil microbial population was most significant during the first 3 days after application. Thereafter, the populations of bacteria, fungi, and actinomycetes increased gradually.

The inhibitory effects of calcium cyanamide on soil borne diseases have been widely studied (Bourbos et al., 1997; Donald et al., 2004; Huang et al., 2006). However, there have only been a few reports of its effects on soil microbial community structure in the presence of growing plants. In our experiment, the soil microbial community structure was significantly different in the CK treatment from that in the NR, PE and SK treatments during the middle growth period of celery. Most of these PLFAs represent either Gram-negative bacteria (Wilkinson, 1988) or fungi (Frostegard and Baath,

1996). Nitrogen competitions exists among soil microorganisms and plants during the growing season when soil N availability is low, leading to N limitation for soil microorganisms (Zak et al., 1990; Hines et al., 2006). At 49 d after fertilization, total N, NO_3^- and NH_4^+ showed no significant differences between the CK treatment and the other treatments. However this does not imply that N was not limited in the CK treatment. Some work has shown that mono-unsaturated PLFAs and fungi indexed PLFAs increased under starvation conditions such as N limitation (Heipieper et al., 1996; Traore et al., 2016), consistent with the current results. Du et al. (2018) found that the abundance of soil bacteria (including nitrifiers and denitrifiers), fungi, and actinomycetes increased with increased N application in greenhouse celery production.

After the celery was harvested, the soil microbial community structure in the PE treatment differed significantly from those in the other three treatments. Compared with the other treatments, the PE treatment had a higher concentration of Gram-positive bacteria (Wilkinson, 1988), and a lower concentration of Gram-negative bacteria and fungi (Frostegard and Baath, 1996; Mutabaruka et al., 2007). Calcium cyanamide used in the PE treatment increased soil pH and supplied enough N during the whole growth period of the celery. However, as, after 49 d fertilization, there was no significant difference in the soil microbial community structure between the PE, NR and SK treatments, we are unable to prove that the differences in PLFA profiles after 78 d fertilization were due to the changes in the soil pH and N content. Besides, the side effects of calcium cyanamide on the majority of microorganisms are negligible after 15 d (Shi et al., 2009). Therefore, the differences are unlikely to be caused directly by toxicity induced by the degradation of calcium cyanamide. There was no significant difference in the growth of celery in any of the treatments from 49 d after fertilization. The yield was obviously higher in the PE treatment than in the other treatments at 78 d after fertilization. As about 40% of plant photosynthates are released into soil from the roots (Lynch and Whipps, 1991), there should be higher nutrient availability in the rhizosphere region of soil in the PE treatment. Differences in the amount of root exudates will affect the survival of different microbial species (Marschner and Timonen, 2006). This might be why the soil microbial community structure is different in the PE treatment compared to the other treatments.

5 Conclusions

We have shown that application of different N fertilizers had different effects on celery yield, soil properties and microbial communities. Calcium cyanamide application significantly increased celery yield, soil pH and soil microbial biomass C, and altered the soil microbial community. Our results suggest that calcium cyanamide is a potential alternative to N fertilizer in acidified vegetable soils. Further work is required to elucidate the subtle changes in detailed species related nutrient cycling and soil-borne diseases using high-resolution techniques.

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Electronic supplementary material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s42832-019-0012-z and is accessible for authorized users.

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