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Effects of Soil C/N Ratio on Apple Growth and Nitrogen Utilization, Residue and Loss

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Abstract Soil C/N ratio is an important influencing factor in soil nitrogen cycling. Two-year old apple trees (Borkh. cv. 'Fuji' / *Malus hupehensis*) were used to understand the effect of soil C/N ratio [6.52 (CK), 10, 15, 20, 25, 30, 35 and 40] on apple growth and nitrogen utilization and loss by using ^{15}N trace technique. The results showed that, with the increasing of soil C/N ratio, apple shoot length and fresh weight increased at first, and then decreased; the higher apple shoot length and fresh weight appeared in C/N = 15, 20 and 25 treatments, and there were no significant differences among these three treatments, but significantly higher than the other treatments. Statistical analysis revealed that there was significant difference in nitrogen utilization rate between the different treatments, the highest N utilization rate was occurred in soil C/N = 25 treatment which value was 22.87%, and there was no significant difference between soil C/N = 25 and C/N = 20 treatments, but both the two treatments were significantly higher than the other treatments; Soil C/N = 40 had the lowest N utilization rate which value was 15.43%, and this value was less than CK (16.65%). The proportion of plant absorption nitrogen from fertilizer was much higher when the value of soil C/N ratio in the range of 15–25, but the percentage of plant absorption nitrogen from soil was much higher when the soil C/N ratio was too low (<15) or high (>25). Amount of residual nitrogen in soil increased gradually with the soil C/N ratio increasing, the amount of residual nitrogen in C/N = 40 treatment was 1.32 times than that in CK. With the increasing of soil C/N ratio, fertilizer nitrogen loss decreased at first, and then increased, fertilizer nitrogen loss was the minimum in C/N = 25 treatments (49.87%) and the maximum were occurred in CK (61.54%). Therefore, regarding the apple growth and nitrogen balance situation, the value of soil C/N ratio in the range of 15–25 would be favorable for apple growth and could increase effectively nitrogen fixed by soil, reduce nitrogen loss, and improve the nitrogen utilization ratio.

Key words Soil C/N ratio, Apple, ^{15}N , Nitrogen utilization, Nitrogen losses

According to statistics, the planting area and output of China's apple in 2011 reached 1.99 million hectares and 35 million tons, respectively, both accounting for more than 50% of the world apple planting area and output. The fruit industry has gradually become a major pillar industry for increasing farmers' income in the apple producing areas^[1]. Nitrogen is the essential macro nutrient for apple's growth and development, and increasing the application of chemical nitrogen fertilizer is one of major measures to increase the apple production^[2–3]. At present, the net nitrogen application rate has reached 400–600 kg · hm² in China's apple orchard, but the nitrogen productivity tends to decline, and the caused environmental pollution tends to aggravate^[4–8]. Therefore, improving nitrogen use efficiency, reducing its negative impact on the environment, and taking into account the ecological benefits while improving apple production, is the guiding ideology for the study of soil nitrogen in China currently^[9]. Soil carbon and nitrogen ratio (C/N) is a sensitive indicator of soil quality, and a indicator for measuring the nutrient balance of soil nitrogen, which has a major impact on the soil nitrogen cycling^[10]. Currently the soil nitrogen content in China's apple orchard has reached as high as 0.8–1.3 g/kg, while the organic matter content is low, only about

1%^[5, 11]. Together with the lack of effective management measures in the ground, the soil C/N ratio is out of balance, which is an important reason for orchard soil quality degradation, decline in soil microbial diversity, serious soil-borne diseases, and decline in fruit quality^[12]. Soil C/N ratio is usually considered a sign of mineralization capacity of soil nitrogen. Low C/N ratio can accelerate microbial decomposition and nitrogen mineralization rates, and high C/N ratio can play a certain role in limiting the soil microbial activity, so that the decomposition and mineralization rate of organic matter and organic nitrogen is reduced and the ability of soil to fixate the organic carbon is increased^[10]. Currently the researches of soil C/N ratio are mainly focused on the tobacco quality and physiological and biochemical indicators and the ways to improve soil C/N ratio by adding organic matter^[13–14], but there are few studies on the effects of C/N ratio on apple plant growth and soil nitrogen cycling.

Therefore, using ^{15}N isotope tracer technique, in this study we research the effects of different soil C/N ratios on the nitrogen balance of apple plant–soil system, in order to provide a theoretical basis for the soil improvement and rational nitrogen application in the apple orchard.

1 Materials and methods

1.1 Experimental design The experiment was carried out in the Horticultural Experiment Station of Shandong Agricultural University. The place features a temperate semi-humid continental monsoon climate. The average annual temperature is 12.9°C, and

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the annual frost-free period is about 195 d. The average annual rainfall is 697 mm and the rainfall is mainly concentrated in June to September. The soil for experiment is brown soil [(organic matter of 10.82 g/kg; total nitrogen of 1.01 g/kg; available phosphorus of 26.21 mg/kg; available potassium of 130.61 mg/kg; pH(H₂O) 6.40]. There are 8 treatments set in this experiment as follows:

- Treatment 1: control (without C), C/N = 6.21;
- Treatment 2: C/N = 10;
- Treatment 3: C/N = 15;
- Treatment 4: C/N = 20;
- Treatment 5: C/N = 25;
- Treatment 6: C/N = 30;
- Treatment 7: C/N = 35;
- Treatment 8: C/N = 40.

Each C/N ratio is regulated by adding different amounts of glucose based on the soil for experiment.

The potted plant is used for this experiment. The pot has diameter of 30 cm and height of 40 cm, and 23 kg of soil is placed into each pot. On March 21, 2012, two-year old apple trees (Borkh. cv. 'Fuji'/Malus hupehensis) were transplanted in the pots, one pot for one plant. 5 pots were prepared for each treatment, and the planting height was 60 cm. After the plants grew steadily, 24 pots of robust pest disease-free plants in the similar way of growing were selected. 3 pots were for each treatment and each pot was a repetition. On April 21, 3 g of 10.25% ¹⁵N - urea (produced from Shanghai Research Institute of Petrochemical Technology), 4 g of superphosphate and 3 g of potassium sulphate were applied to each pot, and watering was immediately done after fertilization.

In the stopping period of plant growth (September 10), the destructive sampling was carried out on the whole plant, to determine the shoot length and plant fresh weight. Then the whole plant was decomposed into roots, stems, and leaves. After the samples were rinsed in the order of water → detergent → water → 1% HCl → 3 times deionized water, they underwent 30 min of water-removing under 105°C, and then they were dried to constant weight at 80°C. After being crushed with electric grinder and sifted through a 60-mesh sieve, they were mixed and bagged for use. All soil in the pot was removed while the plant was decomposed, and after it was weighed and mixed evenly, quartering sampling was used to determine the ¹⁵N abundance of soil samples. ZHT-03 spectrometer (Beijing Analytical Instrument Factory) was used to determine the ¹⁵N abundance of samples.

1.2 Data calculation and statistical analysis Ndff (Contribution rate of ¹⁵N absorbed from fertilizer to be allocated to plant organ to total nitrogen of this organ, %) = [¹⁵N abundance of sample(%) ¹⁵N natural abundance(%)] / [(¹⁵N abundance of fertilizer - ¹⁵N natural abundance(%))] × 100;

Nitrogen use efficiency (%) = [Ndff × total nitrogen of plant organs (g)] / Fertilizer application rate (g) × 100;

Soil ¹⁵N residue = Soil ¹⁵N abundance × Total soil weight ×

soil layer nitrogen content;

Nitrogen residual rate (%) = Soil ¹⁵N residues/fertilizer application rate(g) × 100;

Nitrogen loss rate = 100% nitrogen utilization rate nitrogen residual rate.

Microsoft Excel 2003 was used for drawing tables, and the experimental data used DPS 7.05 for one-factor analysis of variance. LSD method was used for difference significance comparison.

2 Results and analysis

2.1 Effects of soil C/N ratio on plant growth The shoot length and plant fresh weight of apple trees reflect the growth status of plants. As can be seen from Table 1, shoot length and plant fresh weight both increase first and then decrease with the gradual increase in soil C/N ratio. After the exogenous carbon is added to gradually increase the soil C/N ratio to 25, shoot length and plant fresh weight gradually increase to the maximum, then with the further increase in the soil C/N ratio, shoot length and plant fresh weight both gradually decrease. When soil C/N ratio = 40, shoot length and plant fresh weight are both lower than CK.

Statistical analysis results show that the shoot length and plant fresh weight under the three treatments of 15, 20 and 25 are significantly higher than that under other treatments, but there are no significant differences among the three ($P < 0.05$). It shows that adding the exogenous carbon to adjust the soil C/N ratio (15 to 25) is conducive to the growth of apple plants.

Table 1 Effects of soil C/N ratio on apple shoot length and plant fresh weight

Treatment	Shoot length/cm	Fresh weight/g
CK	29.54 ± 1.01 c	212.37 ± 8.34 de
10	33.67 ± 1.79 b	242.56 ± 13.42 c
15	37.89 ± 2.02 a	267.43 ± 11.97 ab
20	38.23 ± 1.27 a	277.75 ± 13.53 a
25	39.43 ± 2.26 a	281.88 ± 16.34 a
30	34.65 ± 1.03 b	261.86 ± 9.27 be
35	30.44 ± 1.29 bc	221.34 ± 12.94 d
40	26.78 ± 1.45 c	197.34 ± 16.82 e

Note: CK means the experiment control without adding the exogenous carbon. 10, 15, 20, 25, 30, 35 and 40 signify the soil C/N ratio, respectively. Data in the same column with different small letters indicate significant difference ($P < 0.05$), the same as follows.

2.2 Effects of soil C/N ratio on plant absorption of fertilizer nitrogen As can be seen from Fig. 1, with the increase in soil C/N ratio, plant's utilization rate of fertilizer ¹⁵N - urea increases first and then decreases. When soil C/N ratio = 25, the plant's utilization rate of ¹⁵N is the greatest, reaching 22.87%, and there is no significant difference between the plant's utilization rate of ¹⁵N when soil C/N ratio = 25 and the plant's utilization rate of ¹⁵N when soil C/N ratio = 20, but the plant's utilization rate of ¹⁵N under both is significantly higher than that under other treatments. When soil C/N ratio = 40, the plant's utilization rate of ¹⁵N is the lowest, only 15.43%, lower than that under CK treatment (16.65%).

The nitrogen absorbed by the plant is on the one hand from the nitrogen provided by the soil, and on the other hand from fertilizer

nitrogen application. Fig. 2 shows that 29.56% – 45.65% of total nitrogen absorbed by the apple plant is from fertilizer nitrogen, and 54.35% – 70.44% of total nitrogen absorbed by the apple plant is from the soil nitrogen, indicating that most of the nitrogen absorbed by the plants is from the nitrogen provided by the soil. When soil C/N ratio is gradually increased to 25, the proportion of total nitrogen absorbed by the apple plants from the fertilizer nitrogen is gradually increased (from 32.67% to 45.65%), and the proportion of total nitrogen absorbed by the apple plants from the soil nitrogen is gradually decreased (from 67.33% to 54.35%).

With the further increase in the soil C/N ratio, the proportion of total nitrogen absorbed by the apple plants from the fertilizer nitrogen is gradually decreased (from 45.65% to 29.56%), and the proportion of total nitrogen absorbed by the apple plants from the soil nitrogen is gradually increased (from 54.35% to 70.44%). It indicates that when the soil C/N ratio is too high (> 25) or too low (< 15), the plant will use more nitrogen in the soil, but absorb less fertilizer nitrogen.

2.3 Effects of soil C/N ratio on ^{15}N residues and losses

As can be seen from Table 2, with the increase in the soil C/N ratio,

the ^{15}N residues will increase in the soil. When the soil C/N ratio = 40, the ^{15}N residue amount and residue rate are both the highest in the soil, 863.89 mg and 28.80%, respectively, 1.32 times the ^{15}N residue amount and residue rate under CK treatment. There is no significant difference in the soil nitrogen residue amount among the treatments with C/N of 25 or more, but significantly higher than the soil nitrogen residue amount under other four treatments.

Compared with CK, the rate of nitrogen losses under all treatments by adding exogenous carbon is reduced. Nitrogen loss rate is gradually reduced and then increased with the increase in the soil C/N ratio. The ^{15}N loss amount and ^{15}N loss rate under the treatment of C/N = 25 is the lowest (1496.08 mg and 49.87%, respectively), only 81.04% of CK. Statistical analysis shows that there are no significant differences in the nitrogen losses between the treatments of C/N = 20 and C/N = 25, but significantly lower than that under other treatments; there are no significant differences in the nitrogen losses among the three treatments of CK, C/N = 10 and C/N = 40, but significantly higher than that under other treatments.

Table 2 Effects of soil C/N ratio on ^{15}N residues and losses in apple – soil system

Treatment	^{15}N residues		^{15}N losses	
	Residue amount // mg	Residue rate//%	Residue amount // mg	Residue rate//%
CK	654.45 ± 22.32e	21.82	1 846.05 ± 41.22a	61.54
10	720.34 ± 16.78d	24.01	1 733.06 ± 39.55ab	57.77
15	783.67 ± 26.81c	26.12	1 573.43 ± 51.56c	52.45
20	802.45 ± 30.22bc	26.75	1 524.35 ± 44.21d	50.81
25	817.82 ± 21.79abc	27.26	1 496.08 ± 31.22d	49.87
30	839.94 ± 24.77abc	27.99	1 583.46 ± 38.31c	52.78
35	848.22 ± 34.11ab	28.27	1 617.48 ± 60.33bc	53.92
40	863.89 ± 18.35a	28.80	1 673.21 ± 29.55abc	55.77

3 Discussions and conclusions

Adding the glucose to improve soil C/N ratio can significantly affect apple plant growth and nitrogen utilization. The studies of Li Xueli *et al*^[14] on tobacco suggest that the soil C/N ratio in 24 – 28 is most conducive to the carbon and nitrogen metabolism during the tobacco plant growth, improving the coordination of internal chemical components of tobacco and improving quality of tobacco, but when the soil C/N ratio is too high, then it is the other way around. The studies of Liu Shiliang *et al*^[15] also show that suitable soil C/N ratio will help improve the content of chlorophyll and nitrogen content and nitrate reductase activity, but with the further increase in C/N ratio, these indicators show a decreasing trend. In this experiment, when the soil C/N ratio is 15 – 25, the plant growth is in the best conditions, and its shoot length and plant fresh weight are significantly higher than that under other treatments, but when C/N ratio is increased to 40, plant's shoot length and fresh weight are even lower than that under the control, which is consistent with the above conclusions. The uptake and utilization efficiency of fertilizer nitrogen also confirms this point.

When the soil C/N ratio is 15 – 25, the plant's utilization rate of ^{15}N is the highest, but with the further increase in C/N ra-

tio, the utilization rate of fertilizer nitrogen is gradually decreased, and when C/N ratio is 40, the fertilizer nitrogen utilization rate is even lower than that under the control treatment without the addition of exogenous carbon. This may be due to the fact that when C/N ratio is high, there is more added exogenous glucose and adding a lot of soluble exogenous carbon promotes the microorganisms in the soil propagate in a large number; the propagation of microorganism need to consume part of nitrogen, and microorganism and apple plant will compete for the nitrogen in the soil, thus affecting the growth of apple plants and roots' absorption of nutrients^[16–17]; when the soil C/N ratio is low, there will be less carbon sources for microbes and microbial activity is decreased, thus affecting the availability of nutrients^[10, 18], making the nutrients difficult to be absorbed by the apple plant roots, thereby inhibiting plant growth and development. From the perspective of apple plant growth and nitrogen uptake, the soil C/N ratio in 15 – 25 is the best.

The nitrogen absorbed by the crops is mainly from the nitrogen provided by soil and nitrogen provided by fertilizer, and soil nitrogen supply is mainly based on mineralization and release of organic matter. Studies have shown that soil mineralized nitrogen

amount is significantly positively correlated with amount of nitrogen absorbed by crops^[19], while soil mineralized nitrogen amount is significantly correlated with the organic carbon level^[20]. Even if in the case of considerable application of nitrogen fertilizer, there is more than about 50% of nitrogen absorbed by crops from the soil^[21].

This study shows that with the increase in the soil C/N ratio, the proportion of nitrogen absorbed by crops from the soil nitrogen supply first decreases and then increases, while the proportion of nitrogen absorbed by crops from the fertilizer nitrogen supply first increases and then decreases. It suggests that low C/N ratio can accelerate microorganisms' decomposition of organic nitrogen in the soil and nitrogen mineralization rate, thereby improving soil's nitrogen supply potential and nitrogen supply capacity^[22-23]. When the C/N ratio is at a relatively high level, it will increase soil microorganisms' "retaining" on the applied fertilizer nitrogen, so that the availability of exogenous nitrogen is reduced, resulting in less absorption of nitrogen fertilizers by the plant. Suitable C/N ratio plays a certain role in limiting soil microbial activity, decelerating the decomposition and mineralization of organic matter and organic nitrogen, which is conducive to improving the soil organic carbon level.

At the same time, it will inhibit the too high intensity of "retaining" of soil microorganisms on the applied nitrogen, which is conducive to giving full play to the effectiveness of nitrogen fertilizer, and promoting the plant's uptake and utilization of nitrogen fertilizers^[10, 18].

The study results in this experiment show that with the increase in the soil C/N ratio, the soil residual nitrogen will increase. Yao Huaiying *et al*^[24] pointed out promoting the microbial fixation of inorganic nitrogen was an effective way to reduce nitrogen losses. Li Shiqing *et al*^[25] confirmed that the application of organic fertilizer can promote microbial nitrogen fixation, and effectively reduce nitrogen and ammonia volatilization losses. The high soil organic carbon content can provide adequate carbon for the microorganisms, helping to increase the number and improve the activity; the excess fertilizer nitrogen that can not be absorbed by the roots, can be assimilated into microbial body to transform into stable organic nitrogenous matter, thereby effectively reducing nitrogen gaseous losses^[26-28]. If the soil C/N ratio is higher, the amount of soil nitrogen fertilizer residues will be greater, but the nitrogen losses first decrease and then increase.

It is mainly due to the fact that when the soil C/N ratio is at a very high level, the proportion of fertilizer nitrogen absorbed by the plants is reduced, and the excessive exogenous carbon makes the soil structure loose, increasing the risk of deep nitrogen leaching losses. In this experiment, the fertilizer nitrogen loss rate under each treatment is in 49.87% - 61.54%, higher than the nitrogen loss rate in the field experiment, which may be related to serious nitrogen leaching losses caused by frequent watering in the summer pot experiment.

From the effects of soil C/N ratio on apple plant growth and

nitrogen balance situation, when the soil C/N ratio is in 15 - 25, it can promote plant growth and development, reduce nitrogen losses and improve fertilizer use efficiency. Therefore, when applying biological straw or exogenous carbon to improve the orchard soil C/N ratio in the apple production, it is necessary to adjust the addition of exogenous carbon and control the soil C/N ratio within the appropriate range, according to the level of soil nitrogen, so as to achieve high yield, high quality and high efficiency.

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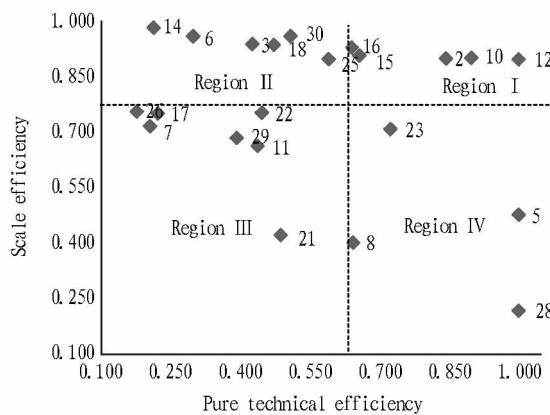


Fig. 1 Distribution of pure technical efficiency and scale efficiency of vegetable specialized cooperatives

For 5 cooperatives in region I, both pure technical efficiency and allocation efficiency are high. Compared with completely effective cooperatives, these cooperatives need make little improvement and may introduce advanced planting technology or adjust scale. For 6 cooperatives in region II, their pure technical efficiency is low, but their scale efficiency is high. These cooperatives should introduce pertinent production technologies, provide training services for cooperative members in vegetable planting, and introduce fine variety cultivation, to improve technical efficiency. For 7 cooperatives in region III, both pure technical efficiency and scale efficiency are low. Firstly, cooperatives should popularize planting technology with the support of government and other organizations. Besides, they should make effort to attract farmers to join in their cooperatives. For 4 enterprises in region IV, the pure technical efficiency is high and scale efficiency is low. To increase overall efficiency, these cooperatives should expand their scale, realize large-scale operation, reduce costs, and increase efficiency.

3 Conclusions and recommendations

Using DEA method and traditional and super efficiency DEA mod-

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els, we analyzed efficiency values of vegetable specialized cooperatives. According to regional division of Chongqing Municipality, we compared efficiency of cooperatives in three regions. Overall technical efficiency, pure technical efficiency and scale efficiency of three regions take on different characteristics, but the overall trend is consistent with local economic development. At the same time, it can be concluded that the super efficiency DEA model can identify and rank order of effective DMUs, so as to make more accurate evaluation on efficiency of cooperatives.

On this basis, it can provide path for judging and analyzing efficiency of cooperatives. We made decomposition of technical efficiency, classified cooperatives with incomplete efficiency, and divided those cooperatives into 4 categories using distribution of pure technical efficiency and scale efficiency. Finally, in line with efficiency distribution of 4 categories of cooperatives, we came up with pertinent measures and recommendations, to point out direction for incomplete effective cooperatives transforming into complete effective cooperatives.

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