Effects of Organic Fertilizer on the Contribution of Nitrogen Resource to Protein, Nicotine and PEE in Tobacco Leaf

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Abstract: Background: Nitrogen is not only an important element for the growth and development of the tobacco plant but also the quality of leaf. The different types of fertilizer have different effect on the contribution of nitrogen resource to protein, nicotine and petroleum ether extract (PEE) in tobacco leaf. **Materials and Methods:** An experiment was conducted with ^{15}N-labeled KNO_3 to study nitrogen sources in tobacco plants and nicotine, protein and PEE in tobacco leaves. Two treatments were designed: chemical fertilizer was only used in treatment I, and chemical fertilizer mixed sesame seed cake was used in treatment Ⅱ. The chemical fertilizer included the ^{15}N-labeled KNO_3. From 68 days after transplant, the plants and leaves were sampled per 10 days, and then atom % ^{15}N excess was determined in both the total N and the three compounds in leaves. **Results and Discussions:** The data showed that the value of N derived from mineral soil N was much more than from fertilizer-N in biomass and the three compounds, and the amount of N from mineral soil was much more in treatment Ⅱ than that in treatment Ⅰ. Although the amount of NO_3-N incorporation into soil was 20% more in treatment Ⅰ than that in treatment Ⅱ, the content of NO_3-N in the biomass was almost same between the two treatments, whereas the other nitrogen forms in the biomass and the three compounds was much more in treatment Ⅱ than that in treatment. Furthermore, the total NO_3-N in protein and petroleum ether extract was obviously lower in treatment Ⅱ than that in treatment Ⅰ, indicating that sesame seed cake fertilizer not only promote N nutrition in soil to be released and utilized, but also improve the utilization rate of NO_3-N.
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Abbreviation: PEE=petroleum ether extract; SSC=sesame seed cake.

1. Introduction

Nitrogen (N) is an essential macronutrient that can frequently act as a limiting factor for plant growth. Nitrogen acquisition of plants is usually dominantly by the uptake of ammonium (NH_4^+) and nitrate (NO_3^-), although soil organic nitrogen can be taken up by plants and may represent a significant proportion of total N absorption under particular ecological situations (acidic soils, low temperature environments). The mineral nitrogen content is generally greater in upper compared with lower soil layers, probably due to more favourable conditions for N mineralization in the upper part of the soil (higher content in organic matter; higher O_2 diffusion).

Because ammonium (NH_4^+) and nitrate (NO_3^-) are fundamentally different in charge, they have quite different effects on the physiological and metabolic processes of plants. Although NH_4^+ is an intermediate in many metabolic reactions, it can result in toxicity symptoms in many plants when it is supplied as the sole nitrogen source.

Nitrogen is not only an important element for the growth and development of the tobacco plant but also the quality of leaf (Zhang et al., 2010). The nitric and carbonaceous compounds should have an adequate ratio in high quality tobacco leaf (Shi et al., 1998). The nitric compounds, especially the contents and the ratio of nicotine, protein and petroleum ether extract in leaf, are crucial factors for the smoking quality (Leffingwell, 1976), and the contents of these compounds have a close relationship with the amount and type of the nitrogen fertilizer (Shi et al.,1998).

It had been reported that the tobacco plant need extra nitrogen nutrient till the flower-bud appearing stage (Zhou et al., 1996). In order to control excessive nitrogen applied to tobacco after the flower-bud appearing stage, the producer always tended to decrease the amount of nitrogen in the field, but the expected efficiency was not attained. It is still a serious problem that the high nicotine levels results in lower quality flue-cured tobacco and lower industrial availability.

In the present paper, to examine the effects of different types of fertilizer on the contribution of

nitrogen resource to protein, nicotine and PEE in tobacco leaf, the experiment with ^{15}N-labeled KNO_3 in chemical fertilizer and SSC mixed with chemical fertilizer was constructed.

2. Material and Methods

2.1 Design of the experiment and tobacco plant materials

The soil was dry fluvo-aquic soil with a pH of 7.1. Although the fertilizer including N (1.44g), P_2O_5 (2.16g) and K_2O (4.32g) was incorporated into 18 Kg of soil per pot, the nitrogen form of fertilizer was different (Table 1), and total nitrogen contained ^{15}N cone 0.73 atom % in treatment Ⅰ and ^{15}N cone 0.44 atom % in treatment Ⅱ. ^{15}N-KNO_3 was supplied by the Chemical Industry Institute of Shanghai.

The tobacco seedling was transplanted on 9th of May, and topping was done on 15th of July. From 68 days after transplant, Leaves, stems, and roots were separately sampled per 10 days. After initial heat treatment at 105 ℃ for thirty minutes, these portions were dried at 60 ℃ and weighed respectively. All of them together were as biomass, then ground into small pieces and passed through a 1 mm sieve. In order to obtain a representative sample for each treatment, three replications per treatment were randomly arranged. The same amount was taken out from the three replications and mixed before it was analyzed.

2.2 Method for the measurement and calculation of nitrogen source

The N % in biomass were analyzed with the method of Nelson and Sommers (1973), and the N in nicotine, protein and PEE were also analyzed with the method of Cao (1980). The atom % ^{15}N excess in biomass and the nitric compounds in leaves ware determined with the Isotope Mass Spectrograph made in America.

atom % ^{15}N excess= ^{15}N %-0.365(nature cone),

$^{15}N_{T1}$:cone 5.239%, $^{15}N_{T2}$:cone 3.150%, $^{15}N_0$: original cone 10.215%,

$^{15}N_{T1}$, $^{15}N_{T2}$ cone=amount of KNO_3×N% in KNO_3×^{15}N cone/N in per pot

Table 1. The design of experiment

Treatment	Rate of SSC-N to total N (%)	SSC-N (g/pot)	NO_3-N (g/pot)	NH_4-N (g/pot)
T1	0	0	0.720	0.720
T2	40	0.576	0.432	0.432

3. Results

Tobacco plant growth, N uptake, amount of mineral soil N, and the contribution of different nitrogen resource to the tobacco plants were closely related to the types of fertilizer incorporated into soil. The growth of tobacco plant in treatment I was poorer than that in treatment Ⅱ during the latter period of tobacco plant growth. Mineral soil N contributing to biomass and the three nitric compounds in treatment I was evidently less than that in treatment Ⅱ (Figure 1), indicating that SSC fertilizer incorporation into soil had a significant effect on promoting nutrition in soil to be released and utilized. It had been reported that SSC fertilizer can modify soil physical properties that can improve the root environment, and consequently enhance the activity of soil enzymes and increase microbiology quantities (Luo and Zhang, 1996). The N accumulation from mineral soil N in tobacco plants and the three nitric compounds was markedly more than that from fertilizer N as given Figure 1.

Protein is the mainly component of cell and catalyzer in plant metabolism, therefore, it would be reasonable that N ratio in protein was similar to biomass (Figure 1a, Figure 1b and Table 2). The changes of N source in nicotine and PEE in leaves were showed in Figure 1c and Figure 1d. There was slight different in N amount from mineral soil N in nicotine and PEE, which increased before day 88, and then declined, but the nicotine remained higher levels in treatment Ⅱ than that in treatment I, and PEE N in treatment Ⅱ was almost the same levels as treatment I after day 98.

The changes of N from fertilizer entering the nicotine and PEE were showed in Figure 1c and Figure 1d. Their changes course between treatments were almost same before day 98, while the amount of nicotine N and PEE N from fertilizer in treatment Ⅱ sharply increased and was more than that in treatment I after 98th day, indicating that mixing SSC fertilizer could improve the fertilizer-N utilization in biosynthesis of nicotine and PEE at the latter stage period. It was suggested that SSC improved much more release of mineral soil N rapidly before day 98, and made the mineral soil N more than the fertilizer-N in soil, resulting that the tobacco plants could absorb more mineral soil N relatively (Figure 1c, Figure 1d and Table 2). Gradually the mineral soil N in the root-zone decreased at the latter stage period, and after day 98 the fertilizer-N in soil in treatment Ⅱ was utilized better than treatment I (Table 3). On the other hand, nicotine and PEE were all the products of secondary metabolism (Trevor, 1974). Generally, their biosynthesis was activity at the latter stage period, so the N assimilated by tobacco plants was supplied preferentially to the synthesis of nicotine and PEE.

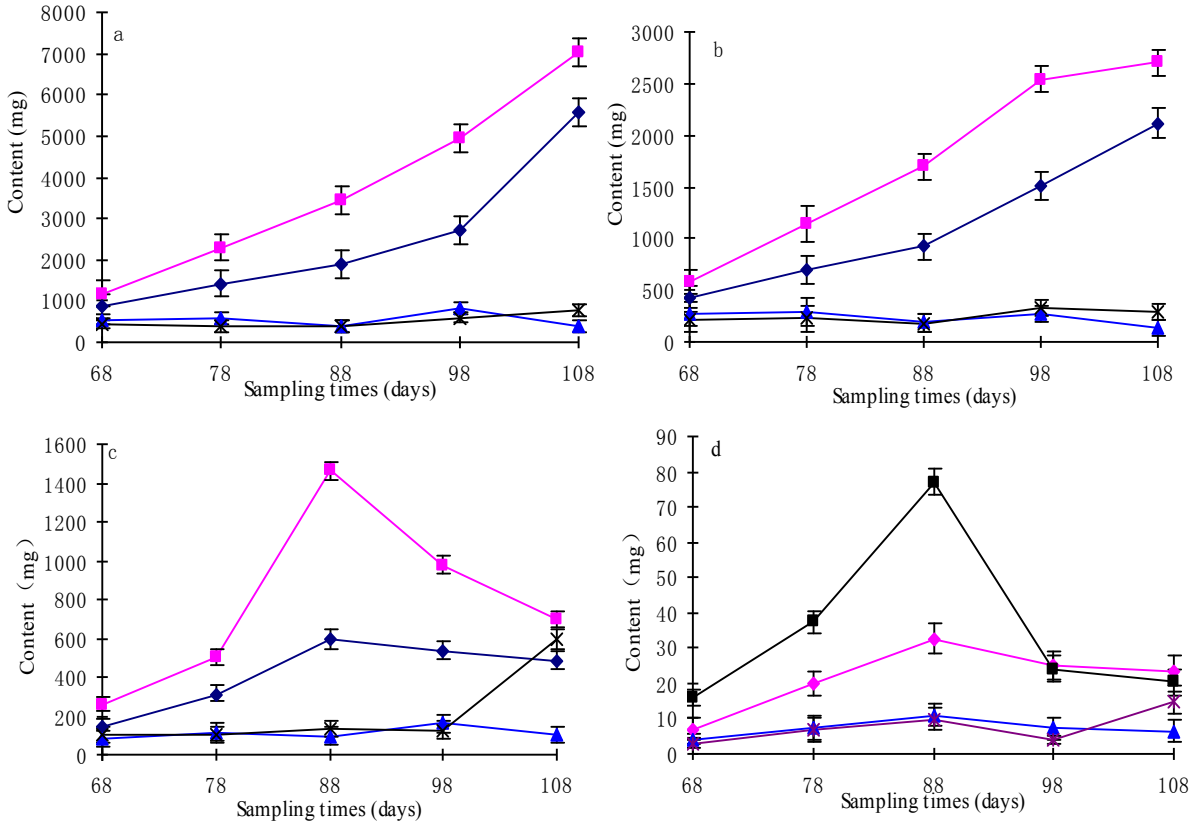

Figure 1. The N resource in biomass (a) ,protein (b) ,nicotine (c) and petroleum ether extract (d) in treatment Ⅰ and treatment Ⅱ,each mean being the average of three replicate pots ,coming from mineral soil N(◆) and fertilizer N (▲) in treatment Ⅰ； coming from mineral soil N(■) and fertilizer N(×) in treatment Ⅱ.

Table 2. Rates of total nitrogen and nitrogen in three nitrogen compounds coming from soil and fertilizer（%）

Treatment			T1					T2		
Day after transplant(d)	68	78	88	98	108	68	78	88	98	108
Total nitrogen (mg)	1412.22	1997.82	2297.85	3533.94	5948.21	1639.82	2683.21	3829.71	5555.41	7818.92
From soil (%)	61.22	70.73	82.97	77.00	93.54	72.03	85.77	90.26	89.12	89.94
From fertilizer (%)	38.78	29.27	17.03	23.00	6.46	27.97	14.23	9.74	10.88	10.06
Protein nitrogen (mg)	697.36	996.69	1108.20	1774.71	2257.24	806.31	1377.29	1874.11	2873.06	2996.34
From soil (%)	60.44	69.93	83.13	84.87	93.81	72.62	83.41	90.42	88.37	90.11
From fertilizer (%)	39.56	30.07	16.87	15.13	6.19	27.38	16.59	9.58	11.63	9.89
Nicotine nitrogen (mg)	226.60	427.84	689.07	697.33	585.61	363.36	602.76	1594.88	1102.41	1292.51
From soil (%)	64.85	72.97	86.35	77.17	82.50	71.68	83.30	91.72	88.59	53.72
From fertilizer (%)	35.15	27.03	13.65	22.83	17.50	28.32	16.70	8.28	11.41	46.28
PEE nitrogen (mg)	11.26	27.24	43.66	32.61	29.94	18.96	44.57	87.02	28.01	35.02
From soil (%)	63.06	73.33	74.86	77.43	78.21	83.60	84.21	88.68	86.19	58.16
From fertilizer (%)	36.94	26.67	25.14	22.57	21.79	16.40	15.79	11.32	13.81	41.84

Table 3. Dynamic change of nitrogen nutrition in soil

Treatment	Day after transplant (d)	Total nitrogen (g)	Atom % ^{15}N excess (%)	^{15}N (mg)	Fertilizer nitrogen (mg)	Mineral nitrogen in root-zone soil (g)
T1	68	15.84	0.055	8.7120	1.6629	15.6840
	78	15.66	0.046	7.2036	1.3750	15.5225
	88	16.56	0.032	5.2992	1.0115	16.4509
	98	16.02	0.333	5.2886	1.0090	15.9191
	108	15.30	0.027	4.1310	0.7885	15.2212
T2	68	15.84	0.052	8.2368	2.6149	15.5785
	78	16.74	0.026	4.3524	1.3820	16.6018
	88	16.02	0.024	3.8836	1.2329	15.9285
	98	16.92	0.022	3.7224	1.1817	16.8018
	108	16.02	0.022	3.5244	1.1189	15.9081

Note: atom % ^{15}N excess=^{15}N%-Soil natural cone (0.236); ^{15}N=Total nitrogen×atom % ^{15}N excess; ^{15}N% left in soil=$^{15}N/^{15}N$ supplied in soil; Fertilizer nitrogen =^{15}N/fertilizer cone $X^{T1:5.239\%;\ T2:3.150\%}$; Mineral nitrogen in soil=Total nitrogen-Fertilizer nitrogen.

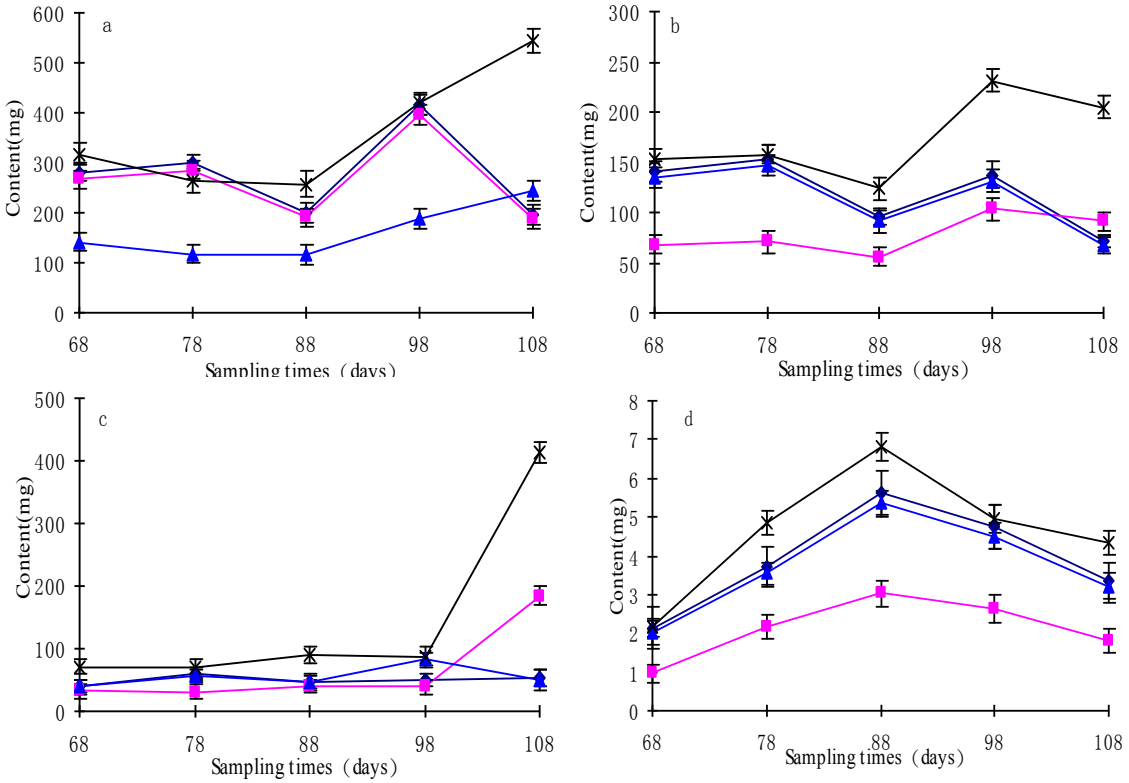

Figure 2. The nitrate-N and other nitrogen forms-N from the fertilizer incorporated into soil in biomass (a), protein(b) nicotine(c),and petroleum ether extract(d) in treatment I and treatment II, each mean being the average of three replicate pots. nitrate-N(◆)and other nitrogen forms-N(▲) in treatment I, nitrate-N(■)and other nitrogen forms-N(✕) in treatment II.

The time courses of changes in the accumulation of NO_3-N and other nitrogen forms from fertilizer in biomass were showed Figure 2a. Between the two treatments, the time courses of changes in NO_3-N accumulated are almost same. It was suggested that the NO_3-N was assimilated and used preferentially in growth of tobacco plant, because the SSC fertilizer

whose nitrogen accounted for 40% in total N incorporation into soil was mixed in treatment Ⅱ. In the other words, the NO_3-N was less 20% in treatment Ⅱ than in treatment I. It was consistent with the conclusion that NO_3-N was easily assimilated and utilized by tobacco plant (Feng and Peng, 1988).

The NO_3-N entering protein or PEE in treatment I had almost the same time course changes as other nitrogen forms, and the former was only little more than the latter. Comparing with treatment I, there was higher amount of other nitrogen forms and lower amount of NO_3-N in treatment Ⅱ (Figure 2b and 2d). The changes in accumulation of N from fertilizer in nicotine were shown in Figure 2c. There was not significant different between NO_3-N and other nitrogen forms in treatment I, but after day 98 the NO_3-N and other nitrogen forms increased sharply in treatment Ⅱ and the other nitrogen forms was one times than NO_3-N at day 108. This suggested that in the latter stage period N assimilated from fertilizer to tobacco plant was more preferentially synthesized into nicotine, the main products of secondary metabolism in tobacco plant. From this point, mixed excessive SSC is not beneficial to the production of high quality tobacco leaves.

4. Conclusion and Discussion

The results of atom $\%^{15}N$ excess of nicotine, protein, PEE and the biomass of the tobacco plants showed that N absorption from soil was much more than that from fertilizer. The total N, nicotine N, protein N and PEE N in tobacco plants grown in the soil mixed with SSC and chemical fertilizer was much more than that in the soil only with chemical fertilizer. Compared with the latter, greater nitrogen mineralization from the former was a result of more N available for mineralization (three times) and a higher percentage of N mineralized from SSC. The application of SSC was also an advantage for enhancing the assimilation and utilization of NO_3-N in the plants.

It was obvious that the amount of nicotine N from the soil was increased transitorily at the day 88 after transplanted, suggesting that the biosynthesis of nicotine had an excellent correlation with topping. In order to benefit the fate of tobacco leaves in the latter stage, top excision must be taken at about 70 days after transplanted, thus accelerating the secondary growth of roots, so the nutrition can be absorbed more easily from the soil by tobacco plants (Han et al., 1998). It was reflected by the kinetic curve line of utilization of soil N by nicotine in Figure 1c and Figure 2c. SSC can enhance the above effect, and offer more N for accumulation of nicotine. However, the latter was not beneficial to the quality of tobacco leaves, because it cannot accord with the N supplying regulation required in tobacco growth. In addition, it is possible that topping can cause a decrease in auxins levels, resulting in active biosynthesis of nicotine (Wang et al., 2008). It had also been observed by Yasurmatsu (1967) that nicotine production was reduced on the addition of auxins to tobacco plants.

A tobacco plant will be physically mature at 98 days after transplanted when the metabolism of synthesis should be lower than the metabolism of decomposition in the plant, so the amount of protein should reduce gradually, compared with before day 98. Whatever the protein nitrogen from soil or from fertilizer in treatment Ⅱ was rather more than that in treatment I (Figure 1b and Figure 2b) , indicating that it was adverse for the maturation of tobacco leaves in the latter stage that the excessive nitrogen of sesame seeds cake released throughout the life of the plant.

The PEE is the aromatic compound in tobacco leaves. The effect of SSC on the nitrogen of PEE can be seen clearly in Figure 1d and 2d. The N, especially from soil, supplied for PEE was more in treatment Ⅱ than treatment I, suggesting that SSC was beneficial to the biosynthesis of PEE. In addition, though NO_3-N content incorporation into soil in treatment Ⅱ was 20% less than that in treatment I, no difference in NO_3-N of nicotine and protein at the day 108 was observed, and the NO_3-N in PEE in treatment Ⅱ was markedly less than in treatment I. This suggests indirectly that NO_3-N was used preferentially in biosynthesis of nicotine and protein in leaves. Further research needs to be done to confirm and expand these preliminary findings with regard to the rate of application of SSC.

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