

Aus dem Institut für Pflanzenernährung und Bodenkunde  
der Christian-Albrechts-Universität zu Kiel

**The effect of plant mineral nutrition on yield and quality  
of green tea (*Camellia sinensis* L.) under field conditions**

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# Catalogues

<b>Abbreviations</b> .....	<b>1</b>
<b>General introduction</b> .....	<b>1</b>
<b>Significance of mineral nutrition for green tea quality</b> .....	<b>2</b>
Nitrogen and the quality of green tea .....	2
Potassium, magnesium and the quality of green tea .....	3
Sulphur and the quality of green tea .....	4
<b>Significance of light intensity for green tea quality</b> .....	<b>5</b>
<b>Aims of the experiments</b> .....	<b>6</b>
<b>Chapter 1</b> .....	<b>9</b>
<b><i>Yield and quality of green tea (Camellia sinensis L.) as influenced by N fertilizer</i></b> .....	<b>9</b>
<b>Abstract</b> .....	<b>9</b>
<b>1. Introduction</b> .....	<b>9</b>
<b>2. Materials and Methods</b> .....	<b>10</b>
2.1. Yield determination .....	13
2.2. Soil chemical properties .....	13
2.3. Mineral nutrient content and quality constituents in tea flushes .....	14
<b>3. Results</b> .....	<b>14</b>
<b>4. Discussion</b> .....	<b>16</b>
<b>Chapter 2</b> .....	<b>21</b>
<b><i>Effects of N supply and interaction between light intensity and N supply on growth and formation of quality-determined constituents of green tea (Camellia sinensis L.)</i></b> .....	<b>21</b>
<b>Abstract</b> .....	<b>21</b>
<b>1. Introduction</b> .....	<b>22</b>
<b>2. Materials and Methods</b> .....	<b>24</b>
2.1. Yield determination .....	24
2.2. Soil chemical properties .....	24
2.3. Mineral nutrient content and quality constituents in tea flushes .....	25
2.4. Determination of ascorbate content .....	25
2.5. Leaf chlorophyll and growth characteristics .....	25
2.6. Extraction and assay of nitrate reductase (NR, EC 1.6.6.1) activity .....	26
2.7. Extraction and assay of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity .....	27
2.8. Extraction and assay of polyphenol oxidase (PPO, EC 1.10.3.1) activity .....	27
2.9. Extraction and assay of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.7), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2) .....	28
<b>3. Results</b> .....	<b>29</b>
3. 1. Responses of yield and quality to shading .....	29
3.2. Mineral nutrient content .....	34
3. 3. Enzyme activities of NR, PAL and PPO .....	36
3. 4. Parameters related to photosynthesis .....	36
3. 5. Ascorbate content and enzyme activities related to ROS detoxification .....	41

<b>4. Discussion</b> .....	<b>42</b>
4.1. Effect of N and shading on yield and quality .....	42
4.2. Effect of shading and nitrogen on enzyme activities of NR, PAL and PPO .....	45
4.3. Effect of shading and nitrogen on photosynthesis.....	48
4.4. Effect of shading and nitrogen on plant antioxidant power .....	50
<b>Chapter 3</b> .....	<b>53</b>
<b><i>Yield and quality of green tea (Camellia sinensis L.) as influenced by N fertilizer and light intensity – possibility to estimate optimum N fertilizer demand</i></b> .....	<b>53</b>
<b>Abstract</b> .....	<b>53</b>
<b>1. Introduction</b> .....	<b>54</b>
<b>2. Materials and Methods</b> .....	<b>56</b>
2.2. Quality constituents in tea flushes.....	56
2.3. Methods to estimate N fertilizer demand .....	56
2.4. Soil and plant samples collection .....	57
2.5. Data analysis of diagnosis.....	57
<b>3. Results</b> .....	<b>58</b>
3.1. Yield and the optimum N application rate.....	58
3.2. N removal and N balance.....	60
3.3. Diagnosis of N status based on yield and quality.....	61
3.3.1. Comparison of methods to estimate N fertilizer demand.....	61
3.3.2. Possible indicators for N fertilizer demand recommendation .....	63
3.3.2.1. Soil N <sub>min</sub> in 0-60 cm soil depth.....	63
3.3.2.2. Total plant N content, NO <sub>3</sub> <sup>-</sup> and amino acid nitrogen (AAN).....	65
3.3.3. Plant indicators and total fresh yield as well as quality.....	67
3.3.4. Plant indicators and soil N <sub>min</sub> in 0-60 cm soil depth .....	74
3.3.5. Critical levels of plant indicators and soil N <sub>min</sub> as well as the optimum N application rate in different seasons.....	78
<b>4. Discussion</b> .....	<b>79</b>
4.1. Yield and the optimum N rate .....	79
4.2. Comparison of indicators .....	80
4.2.1. Responses of plant indicators to soil N <sub>min</sub> .....	81
4.2.2. Responses of yield and quality to plant indicators .....	81
4.3. Critical contents of plant indicators .....	82
4.4. Critical soil N <sub>min</sub> and the optimum N application rate .....	84
<b>Chapter 4</b> .....	<b>89</b>
<b><i>Green tea (Camellia sinensis L.) response to potassium and magnesium fertilization</i></b> .....	<b>89</b>
<b>Abstract</b> .....	<b>89</b>
<b>1. Introduction</b> .....	<b>89</b>
<b>2. Materials and Methods</b> .....	<b>91</b>
<b>3. Results</b> .....	<b>94</b>
3.1. Effects of K and Mg fertilizers on yield and quality elemental contents in flushes and leaves.....	94
3.2. Effect of K and Mg on mineral nutrient content .....	101
3.3. Soil chemical properties.....	105
<b>4. Discussion</b> .....	<b>109</b>

<b>Chapter 5</b> .....	<b>117</b>
<b><i>The effect of sulphur nutrition on the growth and quality of green tea (Camellia sinensis L.)</i></b> .....	<b>117</b>
<b>Abstract</b> .....	<b>117</b>
<b>1. Introduction</b> .....	<b>117</b>
<b>2. Materials and Methods</b> .....	<b>119</b>
<b>3. Results</b> .....	<b>120</b>
3.1. Effect of sulphur on yield and quality.....	120
3.2. Effect of sulphur on mineral nutrient content in flushes.....	121
3.3. Effect of sulphur on the activities of PPO, NR and PAL.....	122
3.4. Effect of sulphur on soil available sulphate and pH.....	123
<b>4. Discussion</b> .....	<b>123</b>
4.1. Responses of yield, quality and mineral nutrient to sulphur.....	123
4.2. Effect of sulphur on enzyme activities.....	124
4.3. Effect of sulphur on the antioxidant protection system of green tea.....	125
4.4. Effect of sulphur on soil available sulphate and pH.....	126
<b><i>General discussion</i></b> .....	<b>129</b>
<b>The effect of interaction between N supply and light intensity on yield and quality</b> .....	<b>129</b>
<b>Diagnosis of N status and the effect of shading on the N diagnosis</b> .....	<b>133</b>
<b>Effect of K and Mg on yield and quality</b> .....	<b>136</b>
<b>Effect of S on yield and quality</b> .....	<b>137</b>
<b>Prospect</b> .....	<b>138</b>
<b><i>Summary</i></b> .....	<b>141</b>
<b><i>Zusammenfassung</i></b> .....	<b>145</b>
<b><i>References</i></b> .....	<b>149</b>
<b><i>Appendix</i></b> .....	<b>161</b>
<b><i>Acknowledgements</i></b> .....	<b>171</b>
<b><i>Curriculum vitae</i></b> .....	<b>172</b>
<b><i>Lebenslauf</i></b> .....	<b>173</b>



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**Abbreviations**

AA:	amino acids
AAN:	amino acid nitrogen
$A_{\max}$ :	light-saturated rate of net CO <sub>2</sub> assimilation at ambient Pa
APX:	ascorbate peroxidase
AsA:	ascorbic acid
$C_i$ :	intercellular CO <sub>2</sub> concentration
DAsA:	dehydroascorbic acid
DHAR:	dehydroascorbate reductase
E:	transpiration rate
GR:	glutathione reductase
$g_s$ :	stomatal conductance
$I_c$ :	light compensation points
LHC:	light-harvesting chlorophyll a/b protein complex
$N_{\text{opts}}$ :	the optimum N application rate within a season
$N_{\text{opty}}$ :	the optimum N application rate within a year
NR:	nitrate reductase
PAL:	phenylalanine ammonia-lyase
Pn:	net photosynthesis
PPO:	polyphenol oxidase
$R_d$ :	dark respiration rate
ROS:	reactive oxygen species
SOD:	superoxide dismutase
tAsA:	total ascorbic acid
$T_L$ :	leaf temperature
TP/AA:	the ratio of tea polyphenols to amino acids
TP:	tea polyphenols
$VPD_L$ :	leaf-to-air vapor pressure difference





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## General introduction

All tea plants come from the *Camellia sinensis* (L.) O. Kuntze bush, a small flowering evergreen, native to China and India which is related to the common camellia. As early as 3000 B. C., tea was used by Chinese as a medicinal drink and as a beverage by the end of the sixth century. The medical use of tea was recorded in the ancient Chinese pharmacopoeia “Ben Cao Gang Mo” written by ShiZheng Li in the Ming dynasty (16<sup>th</sup> century). The first green tea was exported in 1611, when the Dutch East India Company established a factory on Japan’s Hirado Island. Currently, 2.5 million metric tons of tea leaves are produced each year throughout the world, with 20 percent produced as green tea, which is mainly consumed in Asian countries and in some parts of North Africa. Only recently has green tea become more widely available and consumed in the United States. Nowadays, tea is a popular beverage in most countries and its consumption is increasing due to its positive effects on human health (Ganguly, 1993).

During manufacturing of green tea, preservation of the intact green leaf is of utmost importance. Green teas are not fully fermented like black teas, or partially fermented like oolongs. Instead, the tea leaves are plucked, steamed or pan fried (which inactivates the fermentation enzymes, steaming is usually used in Japan, while pan firing is usually used in China), rolled, and then dried. This process yields a chemical composition in green tea similar to the fresh tea leaf. The taste of tea is based on the taste threshold value of chemical components in tea and the reaction of sensory organs to these components. The compounds which play the major role in taste are tea polyphenols, amino acids and polysaccharides (Chen and Yu, 1994). The most important standard for green tea is “freshness and fullness”. The “freshness” is a reflection of amino acids (Wang et al., 1988). The “fullness” is a reflection of the suitable ratio of amino acids and tea polyphenols. Tea polyphenols reflect the characteristic colour of tea infusion, its astringency and unique taste (Hara and Luo, 1995). The colour of green tea is mainly determined by the chlorophyll and some flavone compounds, such as vitexin and isovitexin (Chen and Yu, 1994). Tea aroma constitutes a group of flavour compounds. There are more than 230 identified flavour components in green tea with alcohols and

pyrazines in the greatest proportion. Alcohols are contained in the intact fresh leaves, but pyrazines are formed during the drying process (Chen and Yu, 1994). Caffeine makes a significant contribution to the briskness and creaming properties of tea (Hara and Luo, 1995).

Nutrient deficiency in soils and poor fertilization are possibly two reasons for low yield and quality of tea (Malenga, 1994). With the fast development of tea production in China, great attention has been paid to the balanced fertilization in tea cultivation for good quality and high yield of tea in recent years. It is necessary to study the background of nutrients and the soil properties in tea-grown soils, and their effects on tea quality and yield in order to develop efficient fertilization.

### **Significance of mineral nutrition for green tea quality**

Mineral nutrients are essential to plant growth and development. The beneficial effect of adding mineral elements to soils to improve plant growth has been known in agriculture for more than 2000 years. The growth and development of tea plants, like other plants, also need the continuous supply of mineral nutrition, especially the supply of N, K, Mg and S that are essential to green tea plants. This is because they can affect the content of polyphenols and N-containing compounds and thus affect the quality of green tea.

### **Nitrogen and the quality of green tea**

Nitrogen is normally a key factor in achieving good quality and high yields for green tea. Free amino acids (particularly theanine) in tea flushes plays a major role as quality components of green tea. Among the present identified amino acids, theanine is the most abundant and accounts for 50% of the total amino acids and 1% of the dry weight of tea (Hara and Luo, 1995). The study of biosynthesis of theanine in tea indicated that theanine ( $\gamma$ -glutamylethylamine) is synthesized in the roots from the precursors of glutamic acid (synthesized from ammonium in roots) and ethylamine (synthesized from alanine by alanine decarboxylase and transported down from the shoots) in the presence of L-glutamate: ethylamine ligase (Konishi, 1980 in Morita et al., 1998; Hara and Luo, 1995).

However, the ethylamine formed from the degradation of theanine is partly involved in the synthesis of catechins. Light may be needed for the degradation of theanine (Kito et al., 1968), that is, transformation from theanine to catechin can be restricted by shading. Therefore, theanine is accumulated in shade, which is important to increase the green tea quality.

Modern production of tea requires efficient, sustainable, and environmentally sound management practices. Selection of the most appropriate rate of N fertilizer is one of the management practices that can affect both economic viability of tea production and impact of agriculture on the environment. However, in most commercial tea production, only the importance of nitrogen to increase yield is recognized so that it is usually applied in a large amount simply to reach the high yield, but the negative effect of N overuse is often neglected. The excessive N application is an economic loss to growers in terms of unnecessary input costs and may also result in greater pest problems (Jansson and Smilowitz, 1986; Rossi and Strong, 1991). Moreover, from an environmental perspective, overuse of chemical N fertilizer has been associated with increased levels of nitrate-nitrogen in ground and surface water (Blackmer, 1987 in Krusekopf et al., 2002). To maximize the profitability in a sustainable way, it is important to optimise the efficiency of fertilizer use.

### **Potassium, magnesium and the quality of green tea**

Tea plants are cultivated in many parts of humid and sub-humid tropical regions in acid soils having pH (H<sub>2</sub>O) of 4.5-5.5 (Othieno, 1992; Ranganathan and Natesan, 1985). Most of the tea soils with low pH are highly weathered. However, the gradual weathering and acidification of soils in these regions can accelerate the losses of potassium and magnesium from soils by leaching. The insufficient supply may result in the elemental deficiency in plants. In tea, of the mineral elements available in the mature leaf tissues, potassium comes next to nitrogen and amounts to 1.5 to 2.0 % on a dry matter basis (Krishnapillai and Ediriweera, 1986) and a typical analysis of the components of the two leaves and a bud shows that the mean magnesium content in hybrid seedling tea is 0.19% (Willson, 1975a). It might be expected that the supply of magnesium to tea would not be a

matter of great concern, but the availability of all bases in typical acid tea soils is low and magnesium deficiency has caused some attention in the past.

In China, preliminary studies indicated that Chinese tea soils were insufficiently supplied with potassium and magnesium except nitrogen and phosphorus and that these two nutrients became the main factors restricting the increase of tea yield and quality (Wu and Ruan, 1994). Field and pot experiments had shown that the yields of different types of tea (black, green and oolong tea) responded significantly to potassium and magnesium applications, suggesting that the application of potassium and magnesium is urgently needed in order to improve both yield and quality (Wu and Ruan, 1994). However, the understanding of the effects of potassium and magnesium on tea quality and in particular on the content of free amino acids in tea leaves is very poor. Results from other crops indicated that amino acids accumulated in plants insufficiently supplied with potassium and magnesium due to the inhibition of protein synthesis (Mengel and Helal, 1968; Marschner, 1995). Therefore, the objective of the present study is to investigate the effects of the nutrients of K and Mg on the yield and quality of tea flushes.

#### **Sulphur and the quality of green tea**

Sulphur has had a significant role in the agricultural production thus far. All crops require sulphur to make specific amino acids. Without sulphur, formation of amino acids and various metabolites containing sulphur, protein synthesis and processes required for efficiency from other inputs would be impaired (Messick et al., 1996). The effect of sulphur fertilizers is easily neglected by widely applying the sulphur-containing fertilizers, such as ammonium sulphate (S 24%) and calcium superphosphate (S 12%). When they are applied as nitrogen and phosphorus fertilizers, they improve the sulphur nutrient supply. However, more recently, with the preference of high-concentration fertilizers, non-sulphur-containing and low sulphur-containing fertilizers, such as urea, diammonium phosphate and triple superphosphate are produced and being used to meet crop requirements for nitrogen and phosphorus. By this ammonium sulphate and single superphosphate no longer comprise the large percent of the total nitrogen or phosphorus

fertilizer applied, as they have in previous years. This change causes an increasing deficit in sulphur as crop production climbs.

Another event has affected worldwide demand for sulphur fertilizer consumption and will become of greater importance in the future. Increased environmental concern has prompted reductions in sulphur dioxide emissions which in the past, has benefited crop growth in many countries. Sulphur dioxide from the atmosphere finds its way into the plant through the soil, whether through precipitation or dry deposition. The Environmental Law Institute reports that many industrialized countries have made dramatic reductions in their sulphur dioxide emissions over the last several years. France, Germany, the U.K., and Japan have reduced their contribution of sulphur dioxide by greater than 30% from 1970 to 1985. Sweden has reduced its sulphur dioxide emissions by 70% since 1970 (Messick et al., 1996).

In some countries or regions, sulphur has been the limited factor of crop production. For instance, within Asia, Bangladesh, up to 90% of the agricultural lands may be sulphur deficient, sulphur as the second limited nutrient element immediately after nitrogen (Messick, et al., 1996). India first documented the need for sulphur additions in the 1960's. Since that time, more than 30 crops in different regions of the country are sulphur deficient and have shown responses to added sulphur (Tandon, 1984). China holds a significant requirement for sulphur fertilizer. The deficit for China in 2010 is estimated to be about 4.1 million tons, more than 42% of the entire regional sulphur fertilizer deficit. In Australia, the decrease of rape production is up to 80% and its oil content decreases by 20% (Colton and Sykes, 1992). This explains why the study of sulphur in agricultural practices is being paid more and more extensive attention by agronomists.

### **Significance of light intensity for green tea quality**

It is well documented that the photosynthetic response of a plant is affected by the light intensity at which it is grown (Moss et al., 1961). Strong correlations also exist between the yield of a crop and its light environment (Earley, et al., 1966). In tea, light can affect not only the yield but also the quality by affecting the metabolism of N and carbon,

therefore light can affect tea quality. Tea plants can be cultivated over a considerable range of conditions from temperate climates to hot, humid subtropics and tropics. The optimum mean daily ambient temperature for tea growth is between 20 and 30°C. When the mean ambient temperature is higher than 30°C and light intensity is high, the growth of the tea plant is retarded. The direct effect of shading is to modify the situation of light, airflow, temperature, and humidity as well as to decrease the physical damage of solar radiation; the indirect effect of shading is to minimize the excessive evaporation of water from leaves. However, shading increases the incidence of tea blister blight (*Exobasidium vexans*) due to the shade tree minimizing the solar radiation (Chen and Yu, 1994). Therefore, the benefit and risk analysis of shading are a disputed issue, possibly because of the wide geographical distribution and various climate conditions. It is regarded that shading is necessary in tea areas with a maximum temperature higher than 35°C and relative humidity lower than 40%. The effects of shading on growth, leaf morphology and production have been reviewed in detail by Wang et al. (1998). Similar studies have been carried out in cherimya (Higuchi, et al., 2001), pedunculate oak and beech (Hees, 1997). However, limited information is available on the basic physiological changes brought about by shading and the relationship between photosynthesis and quality in tea plant. The information on the interaction between light intensity and N supply on tea metabolism is even more limited.

### **Aims of the experiments**

Several field experiments were carried out in Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China to focus on the following aspects as shown in Fig. G1: i) improvement of the N management (N demand diagnosis) and development of suitable rapid tests to optimise the N management; ii) effects of interaction of light intensity and N supply on yield and quality of green tea; iii) effect of the supply of K and Mg on the quality-determined constituents of green tea; v) effect of the S supply on the growth and quality of green tea.

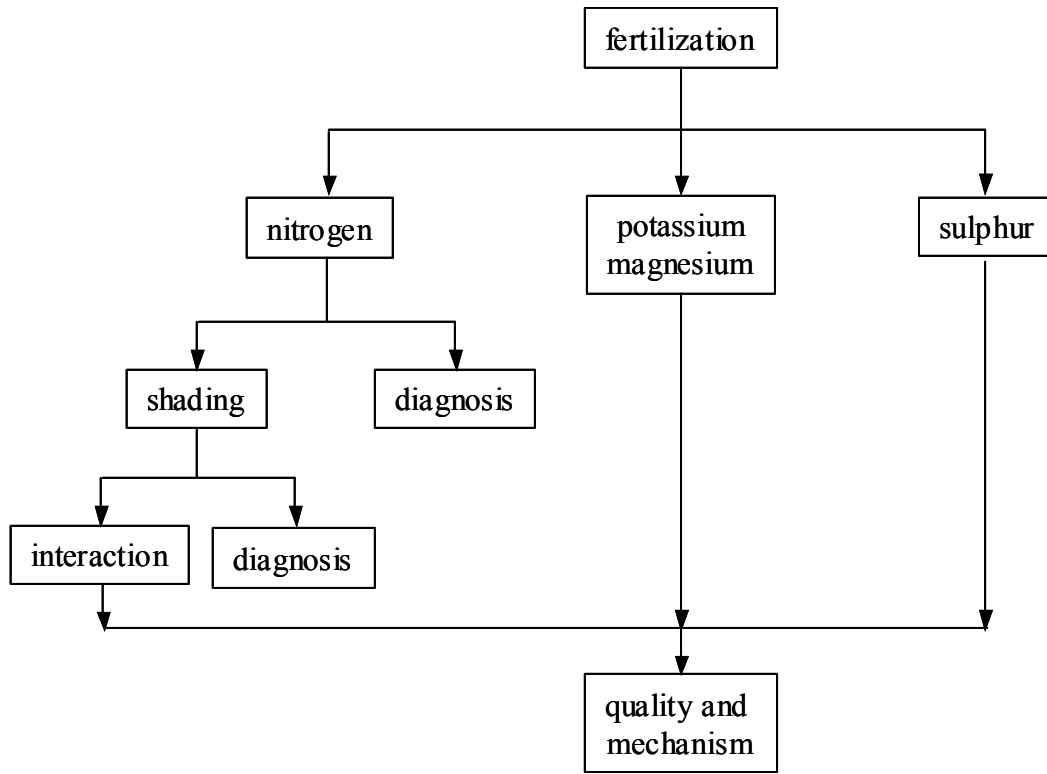


Fig. G1. Aspects which were investigated within this study





## Chapter 1

### Yield and quality of green tea (*Camellia sinensis* L.) as influenced by N fertilizer

#### Abstract

Seven-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Longjing43) in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences (30°16' N and 120°12' E) were used in this study. N field experiment was a 6×4 factorial arranged in a completely randomized block design with 6 N levels (0, 150, 300, 600, 900, 1600 kg N ha<sup>-1</sup> a<sup>-1</sup>) and four replications. It was carried out on Red Soil in 2002 and 2003. No significant responses of yield and quality to N application rate were detected in both years, but yield showed significant difference between both years. Compared to 2002, yield in 2003 showed the increasing trend with ascended N application rate. Quality-determined constituents were hardly affected by N application rate in a season, but by seasons.

#### 1. Introduction

Tea plant (*Camellia sinensis*) originated in the southwest part of China, and has been cultivated for more than 3000 years. At present, tea plants are being cultivated in 54 countries around the world. China is one of eight major tea-producing countries (India, China, Sri Lanka, Kenya, Turkey, Indonesia, former Soviet Union, and Japan) and exported tea from China occupied more than 23% of the international tea market (Wu, 2002). About 70% of tea produced in China is green tea (Chen and Yu, 1994; Wu, 2003). The green tea production accounted for 73% of the whole tea production in China (Wu, 2002). Moreover, with the increased recognition of the advantages of green tea to human health, green tea became one of the three most popular beverages in the world. Its value and importance to human life are attracting more and more attention from agronomist and economist as well as medical scientists.

In commercial production of tea from flushes, there is a continuous removal of nutrients for tea, which are not always adequately supplied in the soil. Their application as fertilizers is therefore recommended annually for sustained high yields (Othieno, 1988) and high quality of tea. Nitrogen is normally a key factor in achieving optimum tea yield and high quality, because nitrogen is markedly and positively correlated with one quality-determined constituent – amino acids (Wang et al., 1988). To maintain economic yields, the application of nitrogen fertilizer is considered necessary (Willson, 1975c). Response to nitrogen fertilizers in tea varies with location, type of fertilizer as well as the type of tea (Owuor et al., 1990). Ordinarily, the schedule of fertilization is determined according to the nutritional status in the soil, the yield level in the previous pruning cycle, and the yield predicted by agro-meteorological conditions. On this basis, the level of nitrogen application is controlled around 240-300 kg per hectare annually in China (Chen and Yu, 1994). However, tea growers applied a large amount of nitrogen into the tea soils in order to achieve high yield. According to the survey in Zhejiang province in China, the N intensity ranges from 279 to 2104 kg N ha<sup>-1</sup> a<sup>-1</sup> (mean 597 kg N ha<sup>-1</sup> a<sup>-1</sup>), while only 214 kg N ha<sup>-1</sup> a<sup>-1</sup> is recommended based on the N removal (12.5-14 kg N kg<sup>-1</sup> made tea) (Ruan and Wu, 2001). The excessive N application is an economic loss to growers in terms of unnecessary input costs and may also result in greater pest management problems (Jansson and Smilowitz, 1986; Rossi and Strong, 1991). From an environmental perspective, overuse of chemical N fertilizer has been associated with increased levels of nitrate-nitrogen (NO<sub>3</sub><sup>-</sup>-N) in ground and surface water (Blackmer, 1987 in Krusekopf et al., 2002). In order to investigate the yield and quality response of green tea to different N application rate, we carried out this experiment with 6 N levels to optimize nitrogen fertilization in green tea production in typical area of green tea production in Zhejiang province.

## 2. Materials and Methods

Seven-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Longjing 43) in the tea garden of Tea Research Institute, Chinese Academy of

Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China (30°16' north latitude and 120°12' east longitude) were used for this study. The field experiment was carried out in 2002 and 2003. The meteorological data in 2002 and 2003 are shown in Table 1.1 and Table 1.2, respectively. Soil type in this site was Red Soil (Ultisol, typic plinthodults) and the chemical properties of soil are shown in Table 1.3. The treatments consisted of different N fertilizer rates (0, 150, 300, 600, 900, 1600 kg N ha<sup>-1</sup> a<sup>-1</sup>), and formed a 6×4 arranged in a completely randomized block design with four replications (Fig. 1.1). Each treatment received a basal application of 150 kg ha<sup>-1</sup> a<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 200 kg ha<sup>-1</sup> a<sup>-1</sup> K<sub>2</sub>O, and 40 kg ha<sup>-1</sup> a<sup>-1</sup> MgO and the resources were Ca-superphosphate, potassium sulphate and magnesium sulphate, respectively. For each treatment, 30% N was applied in the late autumn as the basal fertilizer and another 30% N was applied in the next early spring to improve the germination of the tea buds. After spring, the tea was slightly pruned 12 cm high to maintain good canopy shape. After pruning, 20% N was applied to meet the request of tea canopy growth. After summer, the remaining 20% N was applied to improve the growth of autumn tea.

Table 1.1 The average temperature (T, °C), the highest temperature (HT, °C), the lowest temperature (LT, °C), and relative humidity (RH, %) as well as the monthly accumulated values of precipitation (P, mm), light duration (LD, hours) and evaporation (E, mm) in 2002 (data from Meteorological Station of Hangzhou).

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
T	7.0	9.1	13.6	17.1	20.1	26.1	27.6	26.8	24.1	18.9	12.5	6.9
HT	22.3	19.5	27.5	32.0	32.3	30.7	32.2	30.6	28.4	23.8	17.3	9.9
LT	-0.8	0.5	46.0	8.5	14.5	22.7	24.1	24.0	21.0	15.0	8.9	4.7
RH	42.0	40.0	39.0	45.0	59.0	77.0	80.0	82.0	75.0	74.0	73.0	83.0
P	90.2	56.1	160.9	206.2	153.9	131.7	218	82.8	85.3	87.1	77.0	194.0
LD	154.6	125.7	133.0	81.5	70.7	161.1	143.8	120.6	133.0	149.8	136.0	44.8
E	47.6	40.0	56.9	54.1	50.1	93.3	81.1	86.1	94.9	76.4	45.9	29.5

Table 1.2 The average temperature (T, °C), the highest temperature (HT, °C), the lowest temperature (LT, °C), and relative humidity (RH, %) as well as the monthly accumulated values of precipitation (P, mm), light duration (LD, hours) and evaporation (E, mm) from January to September in 2003 (data from Meterological Station of Hangzhou).

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
T	4.3	7.5	10.7	16.3	21.1	25.0	31.0	29.4	26.1
HT	18.0	23.2	29.7	31.1	32.8	29.4	36.5	34.6	30.8
LT	-4.1	-	-0.1	7.7	11.2	21.4	26.9	25.7	22.5
RH	69.0	-	52.0	60.0	52.0	73	69	74	74
P	41.3	-	172.2	115.4	88.5	116.1	81.4	48.4	43.7
LD	124.4	-	119.1	105.5	123.3	133.1	248.5	196.7	183.7
E	34.5	-	59.6	50.4	81.0	96.6	150.5	133.1	111.2

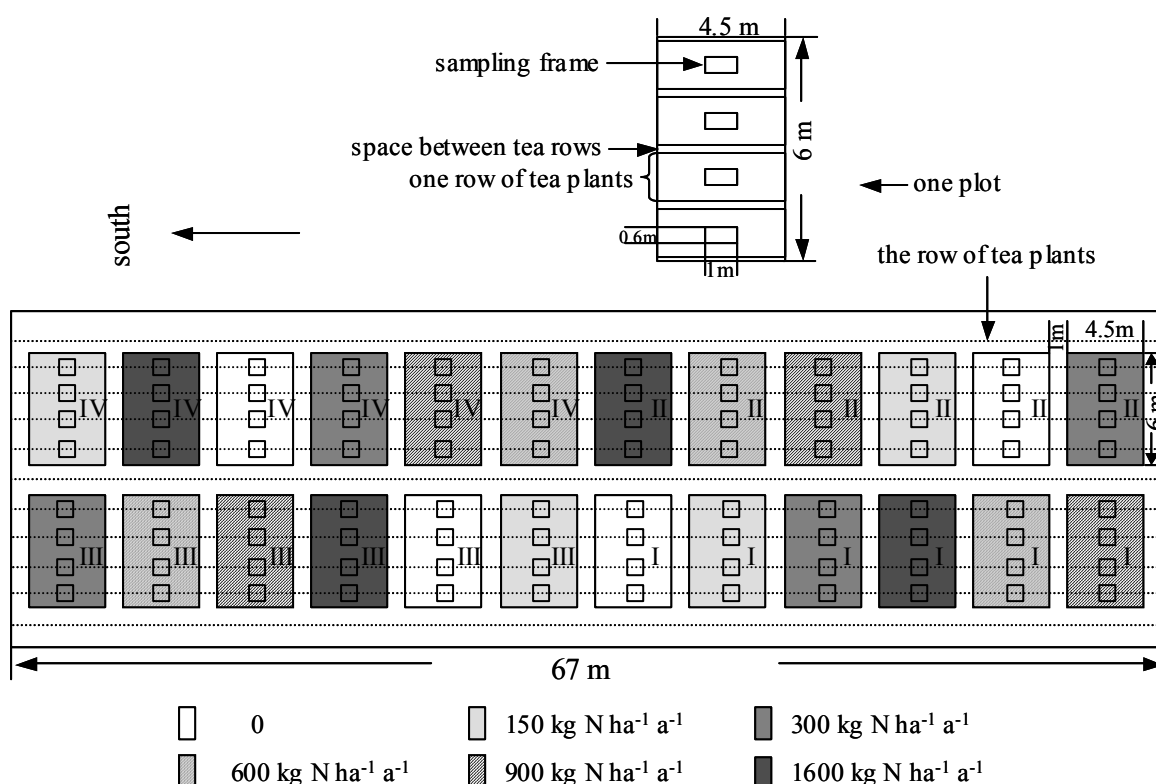


Fig. 1.1 Sketch of sampling way and experimental field set-up in tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China.

### 2.1. Yield determination

Tea flushes in the sampling frame (Fig. 1.1) were taken, weighed and recorded. The fresh yield from the sampling frames within one plot was converted into the fresh yield per hectare. The standards of plucking depended on the seasons. In the early spring, one bud and one leaf were plucked and in the late spring, one bud and two leaves were plucked. After spring tea, the tea flushes were pruned 12 cm high to keep the good shape of canopy, but the pruned tea was not recorded in the yield. In summer, one bud and three or four leaves were only plucked once. In the early autumn, one bud and two leaves were plucked. All of these fresh flushes constituted the annual yield.

### 2.2. Soil chemical properties

The chemical properties of the soil in the experimental site are tabulated in Table 1.3. Soil total N was determined on dried soil by Kjeldahl method (Bremner and Mulvaney, 1982). Soil available P was extracted by the method of dilute acid-fluoride (Olsen and Sommers, 1982), soil exchangeable K and Mg were extracted by the method of ammonium acetate (Thomas, 1982), soil available sulphate was extracted by the calcium phosphate-acid ammonium acetate method with an adaptation of Tabatabai (1982) and Bardsley and Lancaster (1960). The contents of these mineral nutrients in soil were determined with ICP-OES (IRIS/AP). Soil organic matter was measured by the method of Walkley and Black (Nelson and Sommers, 1982) using  $\text{FeSO}_4$  to titrate the excess  $\text{Cr}_2\text{O}_7^{2-}$  after soil organic matter is oxidized by the mixture of  $\text{K}_2\text{Cr}_2\text{O}_7$  and  $\text{H}_2\text{SO}_4$ . Soil  $\text{pH}_{\text{H}_2\text{O}}$  was measured by electric potential method with pH meter (WTW) (Committee of Agricultural Chemistry Specialty, Chinese Soil Academy, 1983).

Table 1.3 The chemical properties of the soil in the site of N experiment.

Soil depth (cm)	Total N (%)	Available P* (mg kg <sup>-1</sup> )	Exchangeable K <sup>‡</sup> (mg kg <sup>-1</sup> )	Exchangeable Mg <sup>‡</sup> (mg kg <sup>-1</sup> )	Available sulphate <sup>§</sup> (mg kg <sup>-1</sup> )	Organic matter (%)	pH <sub>H2O</sub>
0-20	0.02	1.57	92.57	20.59	67.25	2.13	4.56
20-40	0.03	0.44	71.69	18.27	99.12	1.07	4.61

\*: The extractant for P is 0.03 mol L<sup>-1</sup> NH<sub>4</sub>F and 0.025 mol L<sup>-1</sup> HCl;

‡: The extractant for K, Mg is 1.0 mol L<sup>-1</sup> NH<sub>4</sub>OAc;

§: The extractant for SO<sub>4</sub><sup>2-</sup> is 0.01 mol L<sup>-1</sup> Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and 2 mol L<sup>-1</sup> HOAc.

### 2.3. Mineral nutrient content and quality constituents in tea flushes

Quality-determined constituents were measured according to the Tea Research Institute (1983): 1.5 g tea powder was extracted with 250 ml of boiling water in a boiling water bath for 45 min. Total free amino acids were determined with spectrophotometer (UV-752) to detect the blue-purple product from the reaction with ninhydrin at 570 nm (GB8314-87 method) and tea polyphenols were determined with spectrophotometer (UV-752) to detect the purple and blue product from the reaction with iron tartrate at 540 nm (GB8312-87). Caffeine was determined with spectrophotometer (UV-752) to detect the absorbance at 274 nm after the removal of protein, tea polyphenols and pigment and other residues with alkali lead acetic acid and the removal of the excess Pb with H<sub>2</sub>SO<sub>4</sub>.

## 3. Results

It was shown that N application rate had no effect on yield and that only the significant difference between years was detected (Table 1.4). The difference in yield between years was resulted from the different plucking frequency, which was decided by the situation of tea growth and the market requirement. The effect of N levels on increasing yield was minor in 2002 due to the high N residual prior to this study in this site. While in 2003, the differences of yield between treatments were bigger than those in 2002 as shown by the percentage that was actually climbing up with increased N application rates.

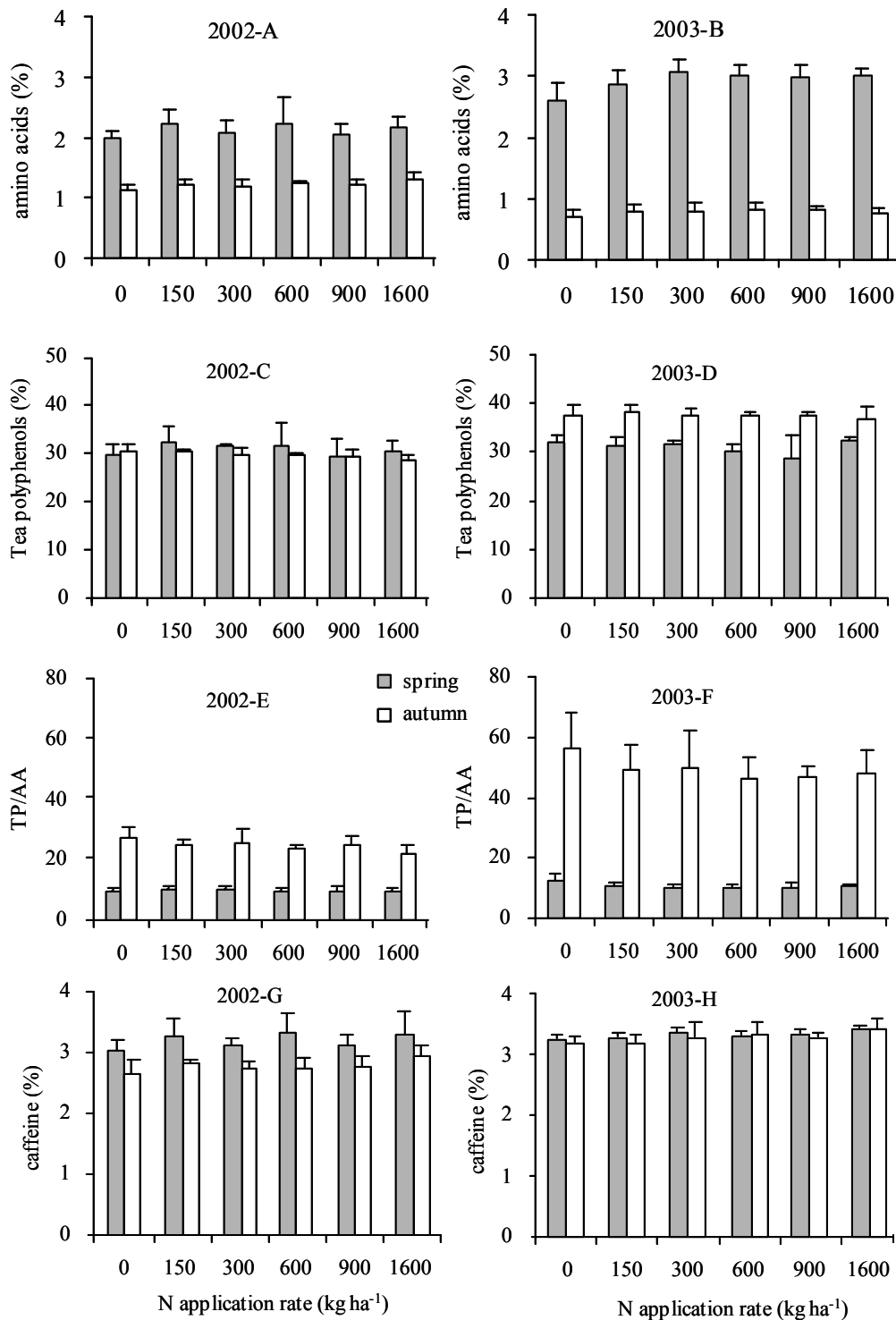


Fig. 1.2 The effects of N levels on tea polyphenols, TP/AA ratio and caffeine content of green tea flushes (one bud and two leaves) in both years.

Summer tea is usually not plucked to make made tea because of its poor quality. Spring is the best growing season for green tea followed by the early autumn, in which the short-time meteorological condition is suitable to green tea production. Thus, only the

results of the quality of spring and autumn tea were shown in Fig. 1.2. It was evident that in both years, the contents of amino acids, tea polyphenols and TP/AA ratio (the ratio of tea polyphenols to amino acids) in tea flushes were not significantly affected by N application rate. Seasons had significant effects on the content of amino acids and TP/AA ratio, especially in 2003. The content of amino acids in spring was significantly higher than that in autumn, while the TP/AA ratio was lower in spring. Tea polyphenols showed different responses to seasons but not to N levels in 2003. In both years, caffeine contents were not affected by either N application rate or season.

Table 1.4 The effect of nitrogen application rate on the yield in 2002 and 2003 (fresh weight kg ha<sup>-1</sup>). The data in brackets were the percentage of increased yield compared to control.

year	N application rate (kg ha <sup>-1</sup> )						Mean
	0	150	300	600	900	1600	
2002	5940	6435±485 (8.3%)	6238±582 (5.0%)	6494±950 (9.3%)	6230±248 (4.9%)	6561±415 (10.4%)	6316B
2003	3923	4139±540 (5.5%)	4333±461 (10.4%)	4328±500 (10.3%)	4700±950 (19.8%)	4798±930 (22.3%)	4370A
Mean	4932a	5287a	5285a	5411a	5465a	5679a	

Small letter: the differences between N application rates.

Capital letter: the differences between years.

#### 4. Discussion

The economic benefit of green tea production is related to yield, quality and regulated by the price of made tea in market. Quality is the major factor to determine the price of tea in market. The quality-determined constituents influence human health by entering food chain. Tea crop consists of leaves, the plant is therefore sensitive to the application of nitrogen fertilizer. Usually, the increase in crop yield is directly proportional to the amount of nitrogen applied. This linear response is found to occur, with few exceptions, for nitrogen application levels up to at least 150 kg ha<sup>-1</sup> (Bonheure and Willson, 1992). At higher nitrogen levels, the response is reduced but remains positive. It was consistent with our results, especially with yield in 2003 (Table 1.4). Although very high nitrogen



application rate may be uneconomic, tea plants are supplied with a large amount of fertilizer nitrogen throughout the growth period (March to September) up to 800-1500 kg ha<sup>-1</sup> a<sup>-1</sup> to achieve high yield and good quality (Tachibana et al., 1996). The surprisingly minor response of the tea crop in our experiments (Table 1.4 and Fig. 1.2) may partly be attributed to the N application rate prior to the onset of our experiment: more than 300 kg N ha<sup>-1</sup> was applied as organic fertilizer (canola seed cake with 6-8% N, 4500 kg ha<sup>-1</sup>) and in addition 100 kg N ha<sup>-1</sup> was applied in mineral form (150 kg ha<sup>-1</sup> urea and 150 kg ha<sup>-1</sup> complex fertilizer NPK) as basal fertilizer and 200 kg N ha<sup>-1</sup> (450 kg ha<sup>-1</sup> urea) was applied as topdressing. Total N in the soil prior to this study was as high as 1900-2000 kg ha<sup>-1</sup>. It has been reported that the total content of amino acids in the first flush was 4% with application of 3000 kg ha<sup>-1</sup> and 3% with application of 1000 kg ha<sup>-1</sup> (Watanabe, 1995), which indicated that the response of quality to N application rate was not sensitive under high N background. The big differences in content of quality-determined constituents between spring and autumn were resulted from the different climate conditions. The changes in content between seasons are regulated by the growth rhythm of tea plant with time. N absorbed by tea plants in spring contributed to the active growth of tea flushes while that in autumn enabled to maintain the mass of tea plant (Watanabe, 1995). This change of N metabolism indicated that N metabolism in spring was active to produce N containing compounds coinciding with growth (such as amino acids and caffeine). When N metabolism slowed down in autumn, carbon metabolic procedure was dominant, preparing for the growth of tea plant in the next spring, and caused the accumulation of containing-C compounds such as tea polyphenols (Fig. 1.2 D, E, F).

N use efficiency (NUE) of tea plant (*Camellia assamica* L.) measured by <sup>15</sup>N-labelled method was 20.0-29.8% in India (Kumar et al., 1999) and 30-37% (*Camellia sinensis* L.) in hilly red soil region in China (Yang, 1991). It was reported that the N amount in pruned tea and withered tree branches as well as fallen leaves was 10-12% of the annual N application rate (Yang, 1991). The N partitioned to tea shoot and roots occupied 10%, while the N removal by plucked tea flushes accounted for 10-15% (Yang, 1991). Under pot culture condition, about 36-45% of the assimilated N was partitioned into the leaves followed by the roots (28-32%) and then the stem (25-31%) irrespective of the different N

levels. On the whole, 40% of the utilized N was harvested as leaves and the remaining 60% was partitioned into other parts of tea plant (Kumar et al., 1999). In our experiment, the N removal by plucking the flushes was only 40-80 kg N ha<sup>-1</sup> a<sup>-1</sup> according to the highest total N content of 6.5%, 4.8% and 4.6% in tea flushes in spring, summer and autumn, respectively (data not shown). Total N removal by plucking and pruned tea based on the highest total N content of 3.5% in pruned tea was 160-200 kg N ha<sup>-1</sup> a<sup>-1</sup>, which was higher than that in black tea production system in Kenya (90-120 kg N ha<sup>-1</sup> a<sup>-1</sup>) (Wanyoko, 1981 in Owuor et al., 1987). The difference in result was mainly because he only reported the N removal by plucking young flushes with 3.0-3.4% of total N content, but without pruned leaves, twigs and branches. It was reported that the mean N content in leaves, twigs and branches was only 0.81% in May (Xun and Zhong, 1997) and 2.80% in leaves (Geng et al., 2001), which are all much lower than 3.5%. Therefore, the total N removal by plucking and pruned tea of 160-200 kg N ha<sup>-1</sup> a<sup>-1</sup> was the maximum value in this study. Moreover, the more important is that the pruned tea was still kept in the field and returned to the production system as the organic matter in our study. Hence, the real N removal from the production system was quite low. The harvesting index (ratio of economic yield to total biological yield) of tea plants according to locations generally ranges from 8% to 15% (Chen and Yu, 1994). It was suggested that the loss of leaching and volatilization of urea was 25.3% in former Soviet Union (Yang, 1991). Moreover, 10-20% of annual N application rate was leached in NO<sub>3</sub><sup>-</sup> form and 10-15% was lost by nitrification and denitrification from tea fields of red soil (Yang, 1991). Denitrification was considered to be the main process to produce N<sub>2</sub>O in acid tea soil with pH value below about 3.60 (Tokuda and Hayatsu, 2000). Considering available N in 0-60 cm soil depth was 50-540 kg N ha<sup>-1</sup> a<sup>-1</sup> (data not shown) depending on N application rates during the experimental period and 1900-2000 kg ha<sup>-1</sup> of total N content prior to this experiment, there was still relatively sufficient N maintained in this production system irrespective of the N loss from it and tea bush was still the main and large N source to meet the flushes growth. The results in this study indicated that two-year time could not exhaust the stored N in tea bush. Therefore, only minor difference was detected by chemical analysis. Meanwhile, this is also one of the reasons for low NUE in this tea production system.

Tea flushes are mainly harvested by hand-plucking in China, the differences between plucking farmers is easily to increase the variance between replications and yield difference between years besides the growth of tea plant, meteorological condition (Table 1.1 and 1.2) as well as market requirement and regulation. The last possible reason is that tea flushes are the most active sink within the growth stage. All the nutrients are first supplied to meet the growth requirement of active growing point. Even when the current N supply in tea soil is not sufficient, the N stored in tea bush still can be transformed to supply the growth of harvesting target. Therefore, there was no detectable big difference in yield and quality of tea flushes between treatments. However, the fact that the percentage of yield increase and amino acid content was gradually climbing up with ascended N levels in 2003 (Table 1.4 and Fig. 1.2 B, F) indirectly demonstrated that nitrogen fertilization virtually had effect on increasing yield and improving quality of tea. Moreover, the apparent difference in growth of tea plants under field condition was observed – tea plants in control showed thinner stems and branches with yellow green leaves and few flushes, while those with N supply showed thicker stems and dense branches with green to dark green leaves depending on N levels. This apparent change in growth of tea plant combined with the minor difference in yield and quality between N levels indicated that N actually affected the growth of tea plant but not the yield of flushes and that the response to any given level of nitrogen can increase year by year for several years at least (Bonheure and Willson, 1992), which therefore also implied that a long-term local experiment is necessary to elucidate the real response of woody plants to N fertilization. Moreover, by this study, it is also urged that N fertilizer efficiency be improved. Most likely fertilizer efficiency could be improved by increasing the  $\text{NH}_4^+$  retention in the soil, and future studies should evaluate the suitability of nitrification inhibitors and identify the proper N application time.



## Chapter 2

**Effects of N supply and interaction between light intensity and N supply on growth and formation of quality-determined constituents of green tea (*Camellia sinensis* L.)****Abstract**

Seven-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Longjing 43) were cultivated in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences (30°16' N and 120°12' E). The experimental design was a completely randomized 6×2×4 design with 6 N application rates (0, 150, 300, 600, 900, 1600 kg N ha<sup>-1</sup> a<sup>-1</sup>), 2 light levels (exposed: 100% full sun and shaded: 36% full sun) and 4 replications by shading for 15 days in summer. The experiments were carried out in 2002 and 2003. Shading reduced the yield, but increased the content of free amino acids and decreased the content of tea polyphenols, which resulted in a lower TP/AA ratio (tea polyphenols to free amino acids). Moreover, the tea flushes under shading had the decreased contents of total ascorbic acid (tAsA), ascorbic acid (AsA) and dehydroascorbic acid (DAsA). Shading increased total contents of P and K in tea flushes. The reduced activities of nitrate reductase (NR) and phenylalanine ammonia-lyase (PAL) under shading might inhibit the synthesis of the precursors of cinnamic acid and catechin, respectively. Moreover, the increased polyphenol oxidase (PPO) in shaded condition might enhance the degradation of polyphenols. The combination of them can partially explain why shading improved the quality of green tea. Under shading, the increased leaf water content, specific leaf area and the decreased ratio of chlorophyll a to chlorophyll b, the leaf total nitrogen content per unit leaf area were the strategies of tea plant to capture more light energy at low irradiance. Decreased light saturated rate ( $A_{max}$ ), light compensation point ( $I_c$ ) and dark respiration rate ( $R_d$ ) under shading explained partially why shading could decrease the yield. The decreased activities of ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and the reduced content

of ascorbate under shading implied that the light driven regulation of the antioxidant and anthocyanin protection system may not be as vital as that in full sun.

## 1. Introduction

Tea (*Camellia sinensis* L.) is the most widely consumed beverage worldwide and has become an important agricultural product (Balentine, 1992 in Benzie and Szeto, 1999). The type and quantity of tea taken varies in different countries and races (Kohlmeier, 1997). However, the close attention paid to the quality of tea is similar. Normally tea quality is greatly influenced by the content of tea polyphenols, amino acids and caffeine in the fresh tea leaves (Cheng, 1983). The quality of green tea is markedly correlated with the contents of total nitrogen and total amino acids, which is a major factor determining the freshness and mellowness of green tea and is positively correlated with the quality of green tea (Wang et al., 1988). Therefore, tea growers have applied increasingly large amounts of nitrogen fertilizers in tea fields. The excessive N application is an economic loss to growers in terms of unnecessary input costs and may also result in greater pest problems (Jansson and Smilowitz, 1986; Rossi and Strong, 1991) and environmental problems (Blackmer, 1987 in Krusekopf et al., 2002).

The summer tea flushes are usually not suitable to make green tea because of the poor quality. In summer, the quality decline of green tea was shown to be caused by the decrease in amino acid content and the increase in polyphenols or catechins, which causes the summer green tea to be bitter and less brisk (Shi and Liu, 1987). Intense light has long been known to disrupt metabolic processes in plants, including photosynthesis, respiration, glucose assimilation, and phosphorylation (Kandler and Sironval, 1959 in Larson, 1988). Shading is the most feasible measure to decrease the high irradiance. The effects of shading on growth, leaf morphology and production have been reviewed in detail by Wang et al. (1998). Shading decreased the leaf area index, delayed the development of flower of peanut and inhibited the productive growth, and the effect of shading on the yield strongly depends on the species. The same results have been reported in trees that shoot length, shoot diameter, leaf number, and total leaf area were reduced under shading, but inter-node length and single-leaf area increased for cherimya (Higuchi, et al., 2001) and

that shading reduced growth, increased partitioning to stem and branches, increased leaf area and reduced partitioning to fine roots of pedunculate oak and beech (Hees, 1997). It has also been well documented in the early literature that the photosynthetic responses of a plant is affected by the light intensity at which it is grown (Burnside and Bohning, 1957; Moss et al., 1961). Considerable attention has therefore been paid to leaf gas exchange and leaf morphology in relation to shading (Crookston, et al., 1975; Zhao and Oosterhuis, 1998; Marengo, et al., 2001; Higuchi, et al., 2001). Their results indicated that specific leaf area was increased and leaf thickness was reduced by shading. While chlorophyll content per unit fresh leaf area was increased. Moreover, under high irradiance, high leaf temperature caused high leaf vapor pressure deficit, resulting in a reduction of gas exchange rate. In spite of these many studies, limited information is available on the basic physiological changes brought about by shading and the relationship between photosynthesis and leaf composition with respect to tea quality. The information on the interaction between light intensity and N on tea metabolism is even more limited.

The reaction catalyzed by nitrate reductase (NR) is the rate-limiting and regulated step of nitrogen assimilation and this enzyme is considered to be a limiting factor for growth, development and protein production in plants (Solomonson and Barber, 1990). Phenylalanine ammonia-lyase (PAL) is the key enzyme of the secondary metabolism (Rühmann et al., 2001). The first committed step for biosynthesis of the phenylpropanoid skeleton in higher plants is the deamination of L-phenylalanine to yield trans-cinnamic acid and ammonia. This reaction is catalyzed by PAL and is often regarded as a key step in the biosynthesis of the flavonoids (Lister and Lancaster, 1996). Polyphenol oxidase (PPO) oxidizes a variety of phenolic substrates, including tyrosine, to L-dihydroxyphenylalanine and then to quinones. Although PPO is considered an essential enzyme during black tea manufacture (Latha and Ramarenthinam, 2000; Ravichandran and Parthiban, 1998), Ye (1992) and Ye et al. (1994) ever did some work on it in green tea. But they simply connected PPO activity with carbon metabolism and did not present any further explanation on that.

This investigation was undertaken to ascertain the effects of interaction between light intensity and N levels on improving the quality of summer tea and its possible mechanism

by measuring the activities of selected important enzymes as well as gas exchange.

## 2. Materials and Methods

In summer, shading experiment was carried out in N experimental field. The experimental design was shown as in Fig. 2.1.

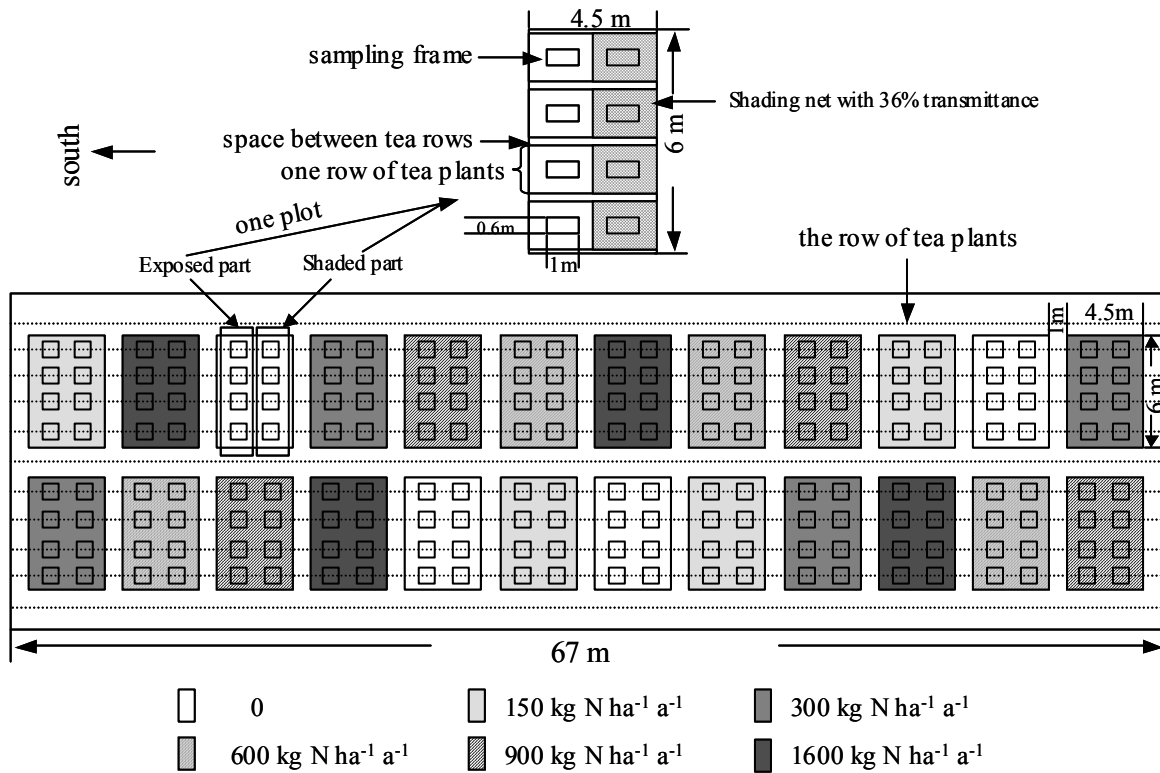


Fig. 2.1 The sketch of the experimental field for shading in Tea Research Institute, Chinese Academy of Agricultural Sciences, P. R. China.

### 2.1. Yield determination

Tea flushes (one bud and two leaves) within the sampling frames in full sun and under shading in each plot were plucked, weighed and converted to the fresh yield per hectare after shading 15 days.

### 2.2. Soil chemical properties

The chemical properties of the soil in the experimental site are tabulated in Table 1.3.



### 2.3. Mineral nutrient content and quality constituents in tea flushes

Total content of N in tea flushes was analyzed by elemental analyzer (EA 1108, Fisons Instrument). Total content of P, K and other elements in flushes was determined by ICP-OES (IRIS/AP) with the pretreatment of dry-ash at 550°C for 5 h and dissolved in 2 ml HCl (1:1) and were diluted to 25 ml with distilled water.

The measurement of quality-determined constituents in tea flushes was identical to Chapter 1.

### 2.4. Determination of ascorbate content

Ascorbate (AsA) and dehydroascorbate (DAsA) were determined by the reformed method according to Cakmak and Marschner (1992). Usually, 1 g fresh tissue was extracted with 10 ml 5% meta-phosphoric acid and centrifuged at 12,000×g for 30 min. Total ascorbate (AsA + DAsA) was measured after reduction of DAsA to AsA with DTT, the concentrations of DAsA were estimated from the difference of total ascorbate (AsA + DAsA) and AsA. The reaction mixture for total ascorbate contained 0.2 ml aliquot of the supernatant, 0.5 ml 150 mmol L<sup>-1</sup> phosphate buffer (pH 7.4) containing 5 mmol L<sup>-1</sup> EDTA, 0.1 ml 10 mmol L<sup>-1</sup> DTT. After 10 min at room temperature, 0.1 ml 0.5% (w/v) N-ethylmaleimide was added to remove excess DTT. The reaction mixture for AsA contained 0.2 ml aliquot of the supernatant, 0.5 ml 150 mmol L<sup>-1</sup> phosphate buffer (pH 7.4) and 0.2 ml water. In both reaction mixtures, the color was developed after addition of the following reagents: 0.4 ml 10% TCA, 0.4 ml 44% ortho-phosphoric acid, 0.4 ml 4% α'-dipyridyl in 70% ethylalcohol, and 0.2 ml 3% FeCl<sub>3</sub>. The mixtures were then incubated at 40°C for 40 min and the colour produced was read at 525 nm.

### 2.5. Leaf chlorophyll and growth characteristics

On 3 Oct. 2002 and 11 Aug. 2003, chlorophyll concentration was quantified from leaf disks obtained from leaves (3<sup>rd</sup> leaf in 2002 and 5<sup>th</sup> leaf which were in the same leaf position with those used to measure photosynthesis in 2003) in each replicate. About 250 mg leaf discs, each 1.0628 cm in diameter, were extracted with 5 ml 80% acetone. The total chlorophyll and chlorophyll a and b were quantified from the absorbance at

652 nm, 645 nm and 663 nm (Bruinsma, 1963). Disc diameter was needed to calculate chlorophyll content per unit leaf area and SLA. Total leaf area was deduced from fresh weight and SLA.

Net photosynthesis (Pn), transpiration rate (E), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) were accomplished with a portable infrared gas analyzer (Li-6400). The light-saturated rate of net CO<sub>2</sub> assimilation at ambient P<sub>a</sub> (A<sub>max</sub>) and the light compensation points (I<sub>c</sub>) were calculated from the above parameters. Measurements were taken on the 5<sup>th</sup> leaf during August in eight randomly selected replicate leaves per treatment after shading 15 days in 2003.

### **2.6. Extraction and assay of nitrate reductase (NR, EC 1.6.6.1) activity**

NR activity was assayed by an adaptation of the method of Wu and Wu (1993) and Chen and Zhang (1980) with spectrophotometer (UV-752) to detect the red coloured reaction product from NO<sub>2</sub><sup>-</sup>, which is formed by the reduction of NO<sub>3</sub><sup>-</sup> by NR, and sulphanilamide as well as N-(1-naphthyl) ethylenediamine hydrochloride at 460 nm. The 3<sup>rd</sup> leaves in 2002 and the 1<sup>st</sup> leaf from the bud were taken as the samples in 2003. All the samples were induced in 50 mmol L<sup>-1</sup> KNO<sub>3</sub> for 12 h at ambient light. After rinsing in distilled water and rubbing dry, the samples were frozen immediately with liquid N<sub>2</sub> and kept in deepfreeze below -26°C until the next early morning. About 1 g samples was ground and extracted with 0.6~0.7 g insoluble PVP, 2 g quartz and 6 ml cold 20 mmol L<sup>-1</sup> HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid) (pH 7.5) containing 5 mmol L<sup>-1</sup> ethylene diamine tetraaceticacid disodiumsalt, 5 mmol L<sup>-1</sup> L-cysteine using mortar and pestle in ice bath. The homogenate was filtered and centrifuged at 10000×g for 20 min at 4°C. The supernatant was used to the assay of NR activity.

The reaction was initiated by the addition of 0.5 ml 100 mmol L<sup>-1</sup> KNO<sub>3</sub> phosphate buffer (pH 7.5), 0.3 ml 0.2 mmol L<sup>-1</sup> NADH (Coenzyme I, reduced, Na<sub>2</sub> salt) and 0.2 ml crude enzyme into tubes (the mixture without NADH as the control). The tubes were incubated at 25°C in water bath for 30 min and the reaction was stopped by the addition of 0.5 ml 1% (W/V) sulphanilamide – 3 mol L<sup>-1</sup> HCl and 0.02% (W/V) N-(1-naphthyl) ethylenediamine hydrochloride. The homogenate was kept still for 15 min and then

estimated by measuring  $A_{540}$  in 10 mm quartz cuvettes. Duplicate assays were performed for each extract. The reactions of 0, 1, 2, 3, 4, 5, 10  $\mu\text{mol L}^{-1}$  standard  $\text{NaNO}_2$  solutions with 0.5 ml 1% (W/V) sulphanilamide – 3 mol  $\text{L}^{-1}$  HCl and 0.02 % (W/V) N-(1-naphthyl) ethylenediamine hydrochloride were used as the standard curve.

### **2.7. Extraction and assay of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity**

PAL activity was assayed with spectrophotometer (UV-752) to determine the amount of cinnamic acid formed per unit time at 290 nm by an adaptation of the method of Cui and Zhang (2003) and the Experimental Manual of Modern Plant Physiology (1999). About 1 g tea flushes (one bud and one leaf) was ground to a fine powder in a mortar and a pestle using liquid  $\text{N}_2$  and 2 g quartz in ice bath. The powder was then extracted with 0.6–0.7 g insoluble PVP and 10 ml 0.1 mol  $\text{L}^{-1}$  Tris- $\text{H}_2\text{SO}_4$  (pH 8.8) extract containing 7 mmol  $\text{L}^{-1}$  mercapto ethanol, 1 mmol  $\text{L}^{-1}$  ethylenediamine tetraacetic acid disodium salt and 7% glycerol. The homogenate was filtered and centrifuged at  $10000\times g$  for 30 min. All these procedures were carried out at or below  $4^\circ\text{C}$  in ice bath. The supernatant was used to the assay of PAL activity.

The reaction was initiated by the addition of 1 ml 0.02 mol  $\text{L}^{-1}$  L-phenylalanine and 2 ml 0.05 mol  $\text{L}^{-1}$  Tris- $\text{H}_2\text{SO}_4$  (pH 8.8) into tubes. The tubes were incubated at  $30^\circ\text{C}$  in water bath for 3 min and then 0.5 ml crude enzyme extract was added. The mixture of 0.5 ml extract solution added served as control. The homogenate was estimated by measuring  $A_{290}$  in 10 mm quartz cuvettes. After the first estimation, the tubes were incubated at  $30^\circ\text{C}$  in water bath for 30 min, and then measured at  $E_{290\text{nm}}$  in 10 mm quartz cuvettes once more. The change of OD values was the real activity of PAL at per unit time. The change of 0.01 OD at 290 nm was one unit of the PAL activity, that is, 1  $\mu\text{g}$  cinnamic acid was formed per 1 ml reaction mixture. Duplicate assays were performed for each extract.

### **2.8. Extraction and assay of polyphenol oxidase (PPO, EC 1.10.3.1) activity**

PPO activity was assayed at 460 nm with spectrophotometer (UV-752) to detect the red coloured oxidation product from catechol by an adaptation of the method of Ye et al. (1994). About 0.5 g tea flushes (one bud and one leaf) was ground to a fine powder with

mortar and pestle using liquid N<sub>2</sub> and 2 g quartz. The powder was then extracted with 0.3 g insoluble PVP and 10 ml buffer with pH 5.6 containing 0.1 mol L<sup>-1</sup> citric acid and 0.2 mol L<sup>-1</sup> di-sodium hydrogen phosphate. The homogenate was incubated for 12 h at 4°C with mixing once every two hours and then filtered and centrifuged at 4000×g for 15 min. All these procedures were carried out at or below 4°C in ice bath. The supernatant was the crude enzyme extract.

The reaction was initiated by the addition of 1 ml crude enzyme and 3 ml reaction mixture containing citric acid-phosphate buffer (pH 5.6), 0.1% L-proline and 1% catechol (mixed by 10:2:3 and citric acid-phosphate buffer instead of 1% catechol in control) into tubes. The tubes were incubated at 37°C in water bath for 10 min and 3 ml 1 mol L<sup>-1</sup> (HPO<sub>3</sub>)<sub>n</sub> was added to terminate the reaction immediately. The absorbance was estimated at A<sub>460</sub> in 10 mm quartz cuvettes. The increase of 0.001 OD at 460 nm was one unit of the PPO activity per unit time.

### **2.9. Extraction and assay of superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC1.11.1.7), dehydroascorbate reductase (DHAR, EC 1.8.5.1) and glutathione reductase (GR, EC 1.6.4.2)**

Usually, 1 g leaf material without the main midrib was homogenized in 10 ml 25 mmol L<sup>-1</sup> EPPS (N-[2-Hydroxyethyl] piperazine-N'-[3-propanesulfonic acid]) buffer (pH 7.8) containing 0.2 mmol L<sup>-1</sup> EDTA and 2% PVP. The homogenate was filtered through a nylon mesh and then centrifuged at 10,000×g for 30 min. The supernatant was used for enzyme analysis. All operations (until analysis) were carried out at 3 to 5°C. With the exception of superoxide dismutase, all enzyme activities were measured in a final volume of 2 ml using various aliquots of the supernatants (100 µL for ascorbate peroxidase, 200 µL for dehydroascorbate reductase and 200 µL for glutathione reductase) (Cakmak and Marschner, 1992).

Activity of **ascorbate peroxidase (APX)** was measured according to Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm ( $E=2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The reaction mixture contained 25 mmol L<sup>-1</sup> phosphate buffer (pH 7.0), 0.1 mmol L<sup>-1</sup> EDTA, 1.0 mmol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub>, 0.25 mmol L<sup>-1</sup> AsA and enzyme aliquot. No change in absorption was

found in the absence of AsA in the test medium. The assay of **dehydroascorbate reductase (DHAR)** was carried out by measuring the increase in absorbance at 265 nm due to AsA formation ( $E=14 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The reaction mixture contained  $25 \text{ mmol L}^{-1}$  phosphate buffer (pH 7.0),  $0.25 \text{ mmol L}^{-1}$  reduced GSH,  $0.4 \text{ mmol L}^{-1}$  DasA and the enzyme aliquot. The reaction rate was corrected for the nonenzymatic reduction of DAsA by GSH. **Glutathione reductase (GR)** was assayed according to the method of Foyer and Halliwell (1976) by following the decrease in absorbance at 340 nm due to NADPH oxidation ( $E=6.2 \text{ mM}^{-1} \text{ cm}^{-1}$ ). The reaction mixture consisted of  $25 \text{ mmol L}^{-1}$  EPPS buffer (pH 7.8),  $0.5 \text{ mmol L}^{-1}$  GSSG,  $0.12 \text{ mmol L}^{-1}$  NADPH and the enzyme aliquot. Corrections were made for NADPH oxidation in the absence of GSSG. **Superoxide dismutase (SOD)** was measured by the adapted photochemical method as described by Giannopolitis and Reis (1977). Assay was carried out on a rotating plate under illumination. The reaction mixture was composed of  $13 \text{ mmol L}^{-1}$  methionine,  $75 \text{ }\mu\text{mol L}^{-1}$  NBT (*p*-nitro blue tetrazolium),  $2.0 \text{ }\mu\text{mol L}^{-1}$  riboflavin  $10 \text{ }\mu\text{mol L}^{-1}$  EDTA- $\text{Na}_2$   $0.05 \text{ mol L}^{-1}$  phosphate buffer (pH 7.8), 0.2 ml distilled water and 0.1 ml crude enzyme extract to bring the final volume to 3 ml. The identical solutions that were not illuminated served as blanks. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of *p*-nitro blue tetrazolium chloride reduction at 560 nm.

All the statistic analysis was done with the GLM procedure (Least Squares Means), SAS (r) Proprietary Software Release (8.02.02MOPO20601), 1999-2001 by SAS institute Inc., Cary, NC. USA.

### 3. Results

#### 3. 1. Responses of yield and quality to shading

The fresh yield results of both summers indicated that shading decreased yield significantly (Table 2.1). In 2002, the fresh yield ranged from 321 to 369  $\text{kg ha}^{-1}$  in full sun, but only 295-314  $\text{kg ha}^{-1}$  under shading. In 2003, the fresh yield was much lower than those in 2002, only 226-276  $\text{kg ha}^{-1}$  in full sun and 146-162  $\text{kg ha}^{-1}$  under shading. Different N levels had no significant effect on yield. No interaction between shading and

nitrogen levels was detected.

Table 2.1 The effects of shading and nitrogen application rate on the yield in summer of 2002 and 2003 year (fresh weight kg ha<sup>-1</sup>).

Year	Light	N application rate (kg N ha <sup>-1</sup> )						Mean
		0	150	300	600	900	1600	
2002	E	321	338	335	323	369	352	340B
	S	295	314	295	301	298	298	300A
	Mean	308a	326a	315a	312a	334a	325a	
2003	E	226	263	245	255	251	276	252B
	S	147	162	148	146	137	153	149A
	Mean	186a	212a	196a	201a	194a	215a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

In 2002, shading significantly increased the content of free amino acids and caffeine and decreased the content of tea polyphenols and TP/AA (the ratio of tea polyphenols to amino acids). Meanwhile, the contents of amino acids and caffeine were increased and the content of tea polyphenols and TP/AA were decreased with the increasing N application rates. Analysis of variance indicated that no interaction between shading and nitrogen levels existed (Table 2.2). In 2003, shading had no significant effects on amino acids, tea polyphenols and TP/AA, but significantly increased the content of caffeine (Table 2.3). Moreover, compared to those in 2002, the quality-determined constituents showed a more significant response to different nitrogen levels, which indicated that the effect of nitrogen application rate was becoming more and more significant with the time going on and/or which also implied that meteorological conditions (Table 1.1 and Table 1.2) had a stronger effect on the results in 2003. However, still no significant interaction was found in 2003 (Table 2.3).

Table 2.2 The effect of shading and nitrogen application rate on the contents of amino acids (AA, %), tea polyphenols (TP, %), the ratio of tea polyphenols/amino acids (TP/AA) and caffeine (Caf., %) in the flushes of green tea after shading 15 days in 2002.

Quality	Light	N application rate (kg N ha <sup>-1</sup> )						Mean
		0	150	300	600	900	1600	
AA	E	1.11	1.22	1.19	1.26	1.20	1.31	1.21A
	S	1.21	1.33	1.37	1.29	1.29	1.30	1.30B
	Mean	1.16a	1.28ab	1.28ab	1.27ab	1.25ab	1.31b	
TP	E	30.35	30.45	29.76	29.46	29.32	28.54	29.65B
	S	26.96	26.24	26.42	27.01	26.02	26.30	26.49A
	Mean	28.66b	28.35ab	28.09ab	28.24ab	27.67ab	27.42a	
TP/AA	E	27.47	25.11	25.44	23.04	24.61	21.97	24.61B
	S	22.41	19.80	19.40	21.02	20.36	20.22	20.53A
	Mean	24.94b	22.45ab	22.42ab	22.02ab	22.48ab	21.10a	
Caf.	E	2.64	2.83	2.74	2.73	2.78	2.94	2.77A
	S	3.11	3.06	3.28	3.16	3.22	3.27	3.18B
	Mean	2.88a	2.94ab	3.01ab	2.95ab	2.99ab	3.10b	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

Table 2.3 The effect of shading and nitrogen application rate on the contents of amino acids (AA, %), tea polyphenols (TP, %), the ratio of tea polyphenols/amino acids (TP/AA) and caffeine (Caf., %) in the flushes of green tea after shading 15 days in 2003.

Quality	Light	N application rate (kg N ha <sup>-1</sup> )						Mean
		0	150	300	600	900	1600	
AA	E	1.23	1.45	1.47	1.68	1.75	1.72	1.55A
	S	1.20	1.38	1.54	1.62	1.67	1.68	1.51A
	Mean	1.21a	1.41ab	1.50bc	1.65cd	1.71cd	1.69d	
TP	E	28.11	26.88	25.17	25.40	25.17	24.54	25.88 A
	S	29.81	27.13	25.06	26.00	25.67	24.24	26.32 A
	Mean	28.96c	27.00bc	25.11ab	25.70ab	25.42ab	24.39a	
TP/AA	E	22.92	18.74	17.22	15.25	14.47	14.57	17.19A
	S	25.01	20.16	16.36	16.06	15.67	14.59	17.97A
	Mean	23.97c	19.45b	16.79ab	15.66a	15.07a	14.58a	
Caf.	E	2.89	2.97	3.08	3.08	3.09	3.08	3.03A
	S	2.93	3.02	3.21	3.24	3.36	3.25	3.17B
	Mean	2.91a	3.00ab	3.14bc	3.16bc	3.23c	3.16c	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).



Table 2.4 The effects of shading and nitrogen application rate on the contents of total ascorbic acid (tAsA,  $\mu\text{mol g}^{-1}$  FW), ascorbic acid (AsA,  $\mu\text{mol g}^{-1}$  FW) and dehydroascorbic acid (DAsA,  $\mu\text{mol g}^{-1}$  FW) in the flushes of green tea after shading 15 days in 2002.

	Light	N application rate ( $\text{kg N ha}^{-1}$ )						Mean
		0	150	300	600	900	1600	
tAsA	E	153.40	156.00	147.86	152.27	164.61	147.25	153.56B
	S	114.16	129.37	113.98	122.23	117.19	127.62	120.76A
	Mean	133.78a	142.68a	130.92a	137.25a	140.90a	137.43a	
AsA	E	105.08	121.29	115.46	120.67	111.50	110.53	114.09B
	S	99.29	108.82	74.21	104.53	103.12	116.13	101.02A
	Mean	102.18a	115.06a	94.84a	112.60a	107.31a	113.33a	
DAsA	E	48.31	34.71	32.40	31.60	53.11	36.72	39.48B
	S	11.87	20.54	39.76	17.70	14.07	11.49	19.74A
	Mean	31.59a	27.63a	36.08a	24.65a	33.59a	24.11a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

Table 2.4 showed that total ascorbic acid, ascorbic acid and dehydroascorbic acid were significantly reduced by shading and that no nitrogen effects and no interaction between nitrogen and shading were found in 2002. In 2003, shading also significantly decreased the contents of total ascorbic acid, ascorbic acid and dehydroascorbic acid, still no nitrogen effect was detected (Table 2.5). Thus there was no interaction between shading and nitrogen application rate.

Table 2.5 The effects of shading and nitrogen application rate on the contents of total ascorbic acid (tAsA,  $\mu\text{mol g}^{-1}$  FW), ascorbic acid (AsA,  $\mu\text{mol g}^{-1}$  FW) and dehydroascorbic acid (DAsA,  $\mu\text{mol g}^{-1}$  FW) in the flushes of green tea after shading 15 days in 2003.

	Light	N application rate ( $\text{kg N ha}^{-1}$ )						Mean
		0	150	300	600	900	1600	
tAsA	E	392.79	361.61	347.28	343.97	320.45	332.63	349.79B
	S	326.68	291.68	299.29	275.33	338.26	270.88	300.19A
	Mean	359.23a	326.65a	323.28a	309.65a	329.35a	301.76a	
AsA	E	312.94	288.35	280.97	273.48	273.50	260.33	281.59B
	S	255.78	246.31	235.92	226.23	273.57	228.03	244.31A
	Mean	294.36a	267.33a	258.44a	249.85a	273.53a	244.18a	
DAsA	E	79.86	73.26	66.31	70.49	46.95	72.30	68.19A
	S	69.89	45.38	63.37	49.11	64.69	42.85	55.88A
	Mean	74.88a	59.32a	64.84a	59.80a	55.82a	57.57a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

### 3.2. Mineral nutrient content

In 2002, shading and nitrogen levels both had no significant effects on the total content of N in tea flushes, but shading could increase the total contents of P, K in tea flushes significantly. However, only total P in tea flushes was significantly affected by nitrogen application rate (Table 2.6). Total contents of Mg, Ca, Al and Mn in tea flushes were all increased significantly by shading but not the nitrogen levels (data not shown). In 2003, shading and nitrogen levels had still no significant effects on total N content in tea flushes, but shading could increase the total contents of P and K in tea flushes and the total contents of P and K were increased with the increasing nitrogen application rates (Table 2.7). Total contents of Mg, Ca, Al and Mn in tea flushes were also increased by shading and no nitrogen effects on them were found except on Al in 2003, too (data not shown). In both years, no interaction between shading and nitrogen was found.

Table 2.6 The effects of shading and nitrogen application rate on the contents of total N, P and K (%) in the flushes of green tea after shading 15 days in 2002.

Element	Light	N application rate (kg N ha <sup>-1</sup> )						Mean
		0	150	300	600	900	1600	
N	E	4.43	4.27	3.91	4.30	4.40	4.35	4.27A
	S	5.33	3.80	4.76	4.57	4.79	3.93	4.51A
	Mean	4.83a	4.03a	4.34a	4.44a	4.59a	4.14a	
P	E	0.46	0.46	0.48	0.48	0.49	0.50	0.48A
	S	0.49	0.50	0.50	0.50	0.50	0.50	0.50B
	Mean	0.47a	0.48ab	0.49ab	0.49ab	0.50ab	0.50b	
K	E	1.20	1.21	1.20	1.20	1.20	1.21	1.20A
	S	1.27	1.28	1.28	1.25	1.27	1.26	1.27B
	Mean	1.23a	1.24a	1.24a	1.23a	1.24a	1.24a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

Table 2.7 The effects of shading and nitrogen application rate on the contents of total N, P and K (%) in the flushes of green tea after shading 15 days in 2003.

Element	Light	N application rate (kg N ha <sup>-1</sup> )						Mean
		0	150	300	600	900	1600	
N	E	4.48	4.54	5.04	5.07	4.62	4.91	4.77A
	S	4.54	4.50	4.50	4.58	4.64	5.14	4.65A
	Mean	4.51a	4.52a	4.77a	4.82a	4.63a	5.02a	
P	E	0.43	0.47	0.48	0.49	0.49	0.49	0.48A
	S	0.46	0.50	0.52	0.52	0.51	0.52	0.50B
	Mean	0.45a	0.49b	0.50b	0.50b	0.50b	0.50b	
K	E	0.58	0.59	0.59	0.59	0.60	0.59	0.59A
	S	0.59	0.60	0.59	0.60	0.60	0.61	0.60B
	Mean	0.59a	0.60ab	0.59ab	0.59ab	0.60b	0.60b	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

### 3. 3. Enzyme activities of NR, PAL and PPO

Shading reduced the NR activity in the 3<sup>rd</sup> leaf in 2002 and the 1<sup>st</sup> leaf in 2003 (Fig. 2.2). But nitrogen application rate had no significant effects on them in both years. Shading significantly decreased the PAL activity and increased the PPO activity in tea flushes under all the nitrogen levels, but the effects of different nitrogen application rate on them were not found in 2003 and thus no interaction between shading and nitrogen levels was found, too (Fig. 2.3).

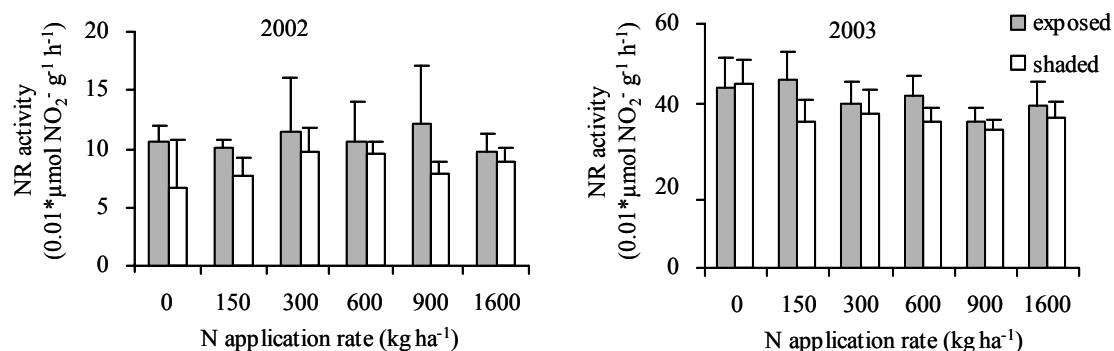


Fig. 2.2 The effect of shading and nitrogen application rate on the nitrate reductase activity after shading 15 days. (the 3<sup>rd</sup> leaf was measured in 2002; the 1<sup>st</sup> leaf was measured in 2003).

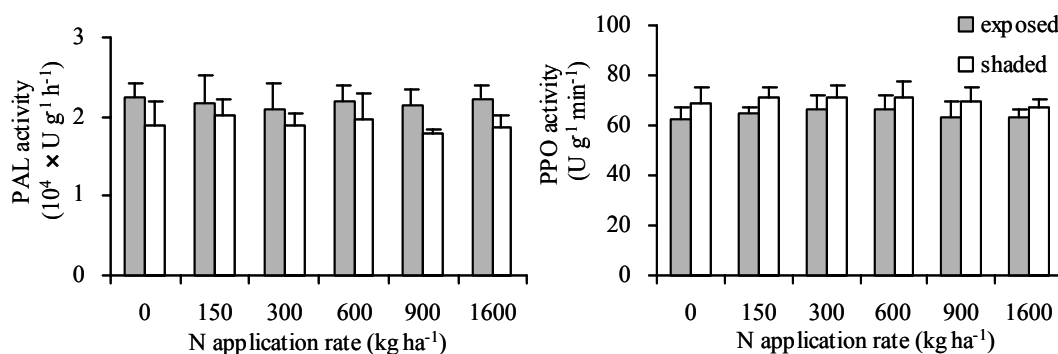


Fig. 2.3 The effect of shading and nitrogen levels on the activities of phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) in flushes (one bud and one leaf) of green tea. PAL ( $\Delta 0.01 \text{OD}_{290} \text{ g}^{-1} \text{ h}^{-1}$ ); PPO ( $\Delta 0.001 \text{OD}_{460} \text{ g}^{-1} \text{ min}^{-1}$ ) in summer of 2003.

### 3. 4. Parameters related to photosynthesis

The leaves subjected to low light intensity maintained higher leaf water content in all the N levels. Thinner and larger leaves developed under shading environments. Single leaf

area was increased and specific leaf area became larger, indicating that leaves in shaded condition reduced leaf thickness in both 2002 (Table 2.8) and 2003 (Table 2.9).

Table 2.8 The effects of shading and nitrogen application rate on water content (%), specific leaf area (SLA,  $\text{m}^2 \text{kg}^{-1} \text{DM}$ ) and chlorophyll a, b (Chl.a, Chl.b,  $\text{g m}^{-2}$ ) and the ratio of chlorophyll a to chlorophyll b (Chla/b) in the new fully expanded leaves of green tea after shading 15 days in 2002 (3<sup>rd</sup> leaf was taken).

	Light	N application rate ( $\text{kg N ha}^{-1}$ )						Mean
		0	150	300	600	900	1600	
WC	E	74.68	74.79	74.78	74.89	74.85	74.53	74.75A
	S	75.27	75.09	75.39	75.30	75.17	75.20	75.23B
	Mean	74.97a	74.94a	75.18a	75.09a	75.01a	74.86a	
SLA	E	15.00	15.92	15.22	14.85	16.16	15.65	15.47A
	S	20.04	18.40	19.15	20.09	19.72	18.83	19.37B
	Mean	17.53a	17.16a	17.19a	17.47a	17.94a	17.24a	
Chl.a	E	0.57	0.59	0.55	0.57	0.56	0.57	0.57A
	S	0.68	0.67	0.79	0.75	0.69	0.70	0.71B
	Mean	0.63a	0.63a	0.67a	0.66a	0.62a	0.64a	
Chl.b	E	0.24	0.25	0.23	0.24	0.25	0.24	0.24A
	S	0.32	0.33	0.42	0.40	0.35	0.35	0.36B
	Mean	0.28a	0.29a	0.33a	0.32a	0.30a	0.29a	
Chla/b	E	2.30	2.36	2.41	2.27	2.30	2.40	2.34B
	S	2.35	2.34	2.23	1.96	1.67	1.76	2.05A
	Mean	2.32bc	2.35c	2.32bc	2.11ab	1.99a	2.08a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

Table 2.9 The effects of nitrogen application rate and shading treatment on water content (WC, %), specific leaf area (SLA, m<sup>2</sup> kg<sup>-1</sup> DM) and chlorophyll a, b (Chl.a, Chl.b, g m<sup>-2</sup>) and the ratio of chlorophyll a to chlorophyll b (Chla/b) in the new fully expanded leaves of green tea after shading 15 days in 2003 (5<sup>th</sup> leaf was taken).

	Light	N application rate (kg N ha <sup>-1</sup> )					Mean	
		0	150	300	600	900		1600
WC	E	73.35	73.34	73.41	73.32	72.83	74.70	73.49A
	S	76.27	76.79	76.12	74.64	75.49	77.06	76.06B
	Mean	74.81a	75.06a	74.76a	73.98a	74.16a	75.88a	
SLA	E	15.22	15.56	15.89	15.12	15.46	16.14	15.57A
	S	20.37	20.99	20.09	19.24	19.82	20.95	20.24B
	Mean	17.80a	18.28a	17.99a	17.18a	17.64a	18.54a	
Chl.a	E	0.37	0.36	0.38	0.40	0.41	0.40	0.39A
	S	0.37	0.38	0.42	0.40	0.43	0.41	0.40A
	Mean	0.37a	0.37ab	0.40ab	0.40ab	0.42b	0.40ab	
Chl.b	E	0.14	0.15	0.15	0.16	0.17	0.16	0.15A
	S	0.15	0.15	0.15	0.16	0.17	0.17	0.16A
	Mean	0.15a	0.15a	0.15ab	0.16abc	0.17c	0.17bc	
Chla/b	E	2.58	2.51	2.60	2.48	2.45	2.43	2.51A
	S	2.42	2.49	2.78	2.52	2.48	2.42	2.52A
	Mean	2.50a	2.50a	2.69a	2.50a	2.46a	2.42a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

Chlorophyll a and chlorophyll b were both increased by shading and the ratio of chlorophyll a to chlorophyll b was reduced significantly by shading in 2002 (Table 2.8). But in 2003, shading had no significant effects on chlorophyll a, chlorophyll b and Chl a/b (Table 2.9). Nitrogen application rate had no significant effects on the contents of chlorophyll a and chlorophyll b, but Chl a/b was decreased with the increasing nitrogen application rates in 2002 (Table 2.8). However, in 2003, though the contents of chlorophyll a and chlorophyll b were increased with the increasing nitrogen levels, no

nitrogen effect on Chl a/b was detected (Table 2.9). The extreme meteorological condition in 2003 might be one of the important reasons resulted in minor effect of shading on Chl a/b. The difference between leaf position in both years might also be one of the reasons.

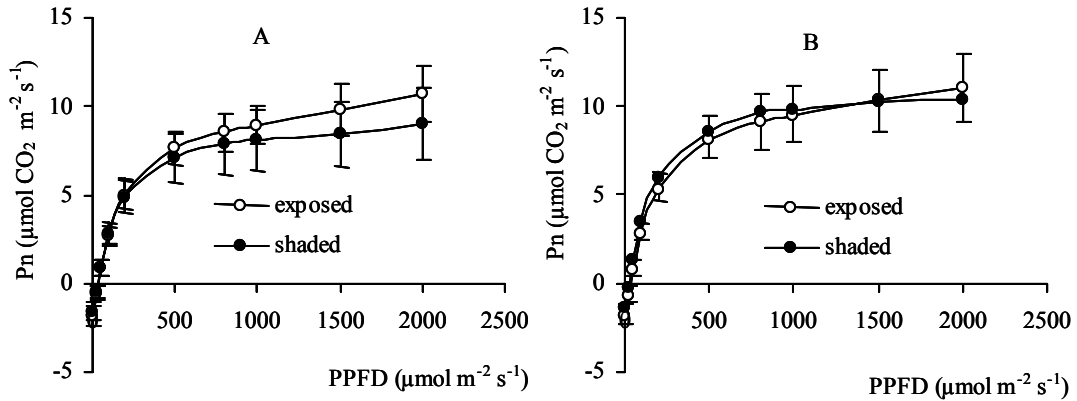


Fig. 2.4 The effect of shading on the net photosynthesis of tea leaves in both nitrogen levels of control (A) and 600 kg N ha<sup>-1</sup> a<sup>-1</sup> (B) in summer of 2003.

The light curves were measured only in two treatments (0 and 600 kg N ha<sup>-1</sup> a<sup>-1</sup>) (Fig 2.4), because the latter represents a typical N application rate. Under control condition, shading inhibited photosynthesis, while in the treatment of 600 kg N ha<sup>-1</sup> a<sup>-1</sup>, the rate of photosynthesis was higher under shading than that in full sun within some extent of PPFD (100 -1500 µmol m<sup>-2</sup> s<sup>-1</sup>). Leaf total N content of shaded leaves in 600 kg N ha<sup>-1</sup> a<sup>-1</sup> was significantly higher than that in control (Table 2.10). Shading could change the leaf total N content in both N application rates. Leaf N content per unit leaf area was reduced by shading in control, but shading had no effect on SLN with the application of 600 kg N ha<sup>-1</sup> a<sup>-1</sup>. Leaf area and specific leaf area under shading were bigger than those in full sun. Leaf water content in shaded leaves was higher than those in exposed leaves. But the SPAD values under shading were not significantly different to those in full sun in both measurements. Light-saturated rate of net CO<sub>2</sub> assimilation at ambient Pa ( $A_{\max}$ ) was 11.09 and 13.12 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in shaded and exposed conditions in control and 12.44 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (shaded) and 13.80 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (exposed) in 600 kg N ha<sup>-1</sup> a<sup>-1</sup>, respectively. In control, shading significantly reduced  $A_{\max}$ , but had a smaller effect on  $A_{\max}$  in the treatment of 600 kg N ha<sup>-1</sup> a<sup>-1</sup>. Light compensation points ( $I_c$ ) were 33.21 µmol

$\text{m}^{-2} \text{s}^{-1}$  under shading,  $36.43 \mu\text{mol m}^{-2} \text{s}^{-1}$  in full sun in control and  $26.21 \mu\text{mol m}^{-2} \text{s}^{-1}$  in shading,  $36.85 \mu\text{mol m}^{-2} \text{s}^{-1}$  in full sun in  $600 \text{ kg N ha}^{-1} \text{ a}^{-1}$  (Table 2.10). Compared to the full sun, shading resulted in a decreased dark respiration rate ( $R_d$ ) on average and nitrogen had no significant effect on  $R_d$ . Shading increased the  $g_s$ , intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and transpiration rate (E), especially significantly increased stomatal conductance ( $g_s$ ) and E compared to the exposed condition in control. Moreover, compared to control, higher N application rate could significantly decrease E under shading.

Table 2.10 Growth and photosynthesis characteristics of tea leaves in summer of 2003 under different light intensities after shading 15 days: total N content (N, %); leaf area (LA,  $\text{cm}^2$ ); specific leaf N content (SLN,  $\text{g N m}^{-2}$ ); specific leaf area (SLA,  $\text{m}^2 \text{ kg}^{-1}$  dry matter); leaf water content (LWC, %); chlorophyll content (SPAD); light-saturated rate of net  $\text{CO}_2$  assimilation at ambient Pa ( $A_{\text{max}}$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); light compensation point of net  $\text{CO}_2$  assimilation at ambient Pa ( $I_c$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); dark respiration rate ( $R_d$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); average stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ); average intercellular  $\text{CO}_2$  concentration ( $C_i$ ,  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ ); average transpiration rate (E,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ).

	N application rate ( $\text{kg N ha}^{-1}$ )			
	0		600	
	shaded	exposed	shaded	exposed
N	3.19aA	3.65aA	3.92bA	3.86aA
LA	14.25aB	12.39aA	14.70aB	12.77aA
SLN	1.77aA	2.15aB	2.78bA	2.73bA
SLA	18.08aB	13.24aA	18.45aB	14.35aA
LWC	73.31aB	70.53aA	74.11aB	70.69aA
SPAD	54.43aA	60.40aA	60.69aA	61.98aA
$A_{\text{max}}$	11.09aA	13.12aB	12.44aA	13.80aA
$I_c$	33.21aA	36.43aA	26.21aA	36.85aA
$R_d$	1.68aA	1.78aA	1.45aA	1.94aA
$g_s$	0.23aB	0.15aA	0.23aA	0.17aA
$C_i$	301.38aA	299.83aA	312.63aA	300.68aA
E	5.35bB	3.69aA	4.14aA	3.46aA

Small letter: the differences between nitrogen levels within each light level ( $p < 0.05$ ,  $n=8$ );

Capital letter: the differences between shaded and exposed within each N application rate ( $p < 0.05$ ,  $n=8$ ).



### 3. 5. Ascorbate content and enzyme activities related to ROS detoxification

Ascorbic acid content in 5<sup>th</sup> leaf was reduced significantly by shading except dehydroascorbic acid. Total ascorbic acid content was significantly decreased with the increasing nitrogen application rates, but it had no significant effect on ascorbic acid and dehydroascorbic acid in 2003 (Table 2.11). Compared to the flushes (Table 2.5), the ascorbate in 5<sup>th</sup> leaf was much higher. The enzymes related to reactive oxygen species (ROS) detoxification or similar were also decreased by shading except superoxide dismutase (SOD). Shading significantly decreased the content of ascorbate peroxidase (APX) and dehydroascorbate reductase (DHAR). The activities of both enzymes were increased with increasing N application rates, especially for APX (Fig. 2.5). But this trend could not always be seen in glutathione reductase (GR). Only the interaction between shading and nitrogen application rate in APX was detected.

Table 2.11 The effects of shading and nitrogen application rate on the contents of total ascorbic acid (tAsA,  $\mu\text{mol g}^{-1}$  FW), ascorbic acid (AsA,  $\mu\text{mol g}^{-1}$  FW) and dehydroascorbic acid (DAsA,  $\mu\text{mol g}^{-1}$  FW) in mature leaves (5<sup>th</sup> leaf) of green tea after shading 15 days in 2003.

	Light	N application rate ( $\text{kg ha}^{-1}$ )					Mean	
		0	150	300	600	900		1600
tAsA	E	517.7	469.3	476.7	439.6	477.0	468.1	474.7B
	S	429.1	387.1	364.7	332.8	378.3	352.2	374.1A
	Mean	473.4b	428.2ab	420.7ab	386.2a	427.7ab	410.2a	
AsA	E	375.4	368.8	351.1	347.5	360.2	368.0	361.8B
	S	300.3	301.5	271.0	263.6	290.7	254.6	280.3A
	Mean	337.8a	335.2a	311.0a	305.6a	325.4a	311.3a	
DAsA	E	142.3	100.4	125.6	92.2	116.8	100.1	112.9A
	S	128.8	85.7	93.7	69.2	87.7	97.6	93.8A
	Mean	135.6a	93.0a	109.7a	80.7a	102.2a	98.8a	

E: exposed; S: shaded.

Small letter: the differences between N application rates ( $p < 0.05$ ,  $n=4$ );

Capital letter: the differences between shaded and exposed ( $p < 0.05$ ,  $n=4$ ).

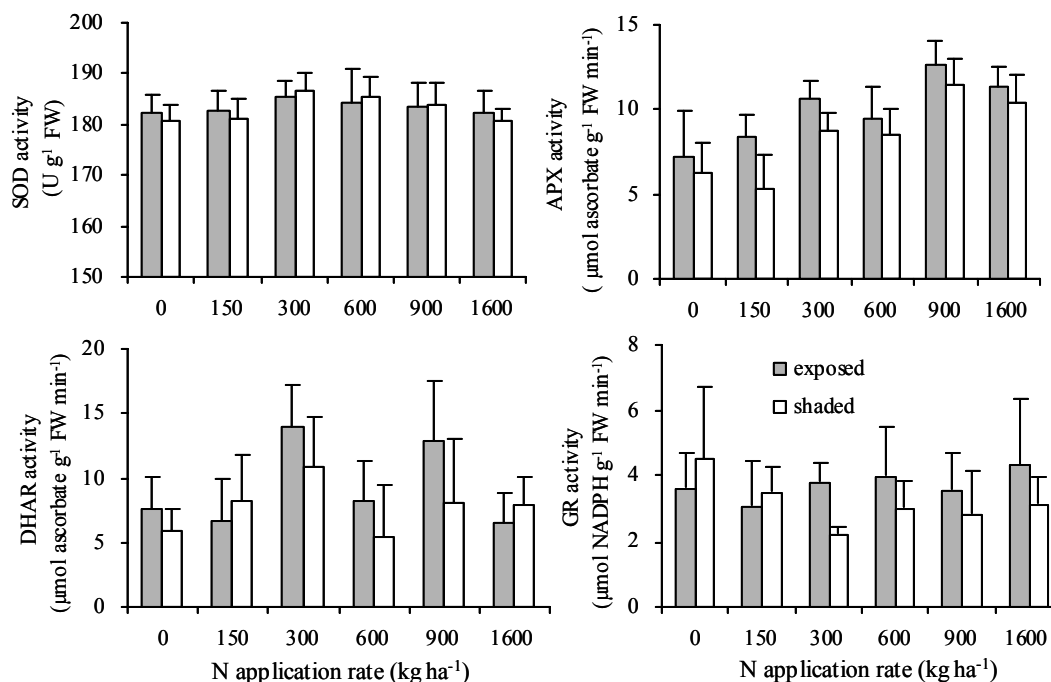


Fig. 2.5 The activities of enzymes related to reactive oxygen species, superoxide dismutase (SOD), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) in the 5<sup>th</sup> leaf in different N application rate and both exposure treatments after shading 15 days.

## 4. Discussion

### 4.1. Effect of N and shading on yield and quality

It has been well known that shading can decrease yield and improve quality of green tea under growth conditions with high temperature and high irradiance. Generally, our results verified this finding (Tables 2.1-2.3). In 2003, the marginal improvement of quality by shading might result from the still high transmitted light intensity after shading with shading cloth in the extreme high irradiance in summer of 2003 (Table 1.1 and Table 1.2). But other quality-determined constituents such as caffeine (Table 2.3) and water extracts (Fig. A8) in 2003 and all of them in 2002 (Table 2.2) indicated that shading had its positive effect on improving the quality (Fong and Shyu, 1988). Increased amino acids and decreased tea polyphenols resulted in the decreased TP/AA ratio, which showed significantly negative linear regression with green tea quality (Shi and Liu, 1987). A study in cotton (Zhao and Oosterhuis, 1998) indicated that shading had the largest effect on

decreasing the total non-structural carbohydrate concentration (hexose, sucrose and starch) in the leaves, which is one of the reasons why shading can decrease the content of tea polyphenols, because the precursor of tea polyphenols is sugar and tea polyphenols are thought to be the compounds containing carbon in a large amount, which include flavanols, flavadiols, flavonoids and phenolic acids; these compounds may amount for up to 30% of the dry weight (Lin et al., 2003). It has been well documented that tea polyphenols with antioxidant properties can prevent oxidative damage (Zhang and Shen, 1997; Benzie and Szeto, 1999; Sawai and Moon, 2000). But in these studies, most of them only elaborated the function or mechanisms of the antioxidative and radical-scavenging activities of catechin. Light is essential for plants, since photosynthetic pigments efficiently absorb visible wavelengths and use their energy for carbon fixation; but sunlight also contains energetic, short-wavelength ultraviolet (UV) photons which are potentially detrimental because of their destructive interactions with many cellular molecules, such as the amino acids of essential proteins, nucleic acid bases, or membrane lipids (Larson and Berenbaum, 1987 in Larson, 1988). Caldwell et al. (in Larson, 1988) have pioneered a concept that flavonoids, with their strong absorption in the 300-400 nm UV region, are acting as internal light filters for the protection of chloroplasts and other organelles from UV damage. They have demonstrated that many UV-absorbing compounds occur in high concentrations in the vacuoles of epidermal cells as well as within chloroplasts. He thought the light-filtering ability of these compounds might reinforce their powerful antioxidant effects to provide a high level of protection against damaging oxidants generated either thermally or by light. Early work on flavonoid content of alpine plants suggested that their concentrations increased with altitude (Shibata and Kishida, 1915 in Larson, 1988); it has also been reported that some red-leaved plants such as *Oxyria diagyna*, a common species in arctic and alpine ecosystems, are very resistant to UV-B (Billings and Mooney, 1968 in Larson, 1988). This may reflect a protective effect of high concentrations of anthocyanin pigments, which are flavonoids. This can exactly explain why the tea flushes are red in summer and this is also one of the reasons why the summer made tea can not have bright tea infusion. Water extracts (Fig. A8) were decreased by shading, which means that the tea flushes grown under shading were more tender than

those grown in full sun, because the lower content of water extracts means the tea flushes contain less water-soluble matter and more organic components.

Vitamin C (ascorbic acid and dehydroascorbic acid) is an indicator of fresh and high quality green tea (Liang et al., 1996). It is widely recognized as a major antioxidant in biological systems, and it has been reported that vitamin C can synergistically regenerate vitamin E, resulting in an enhancement of antioxidant activity in membranes, thereby preventing them from being oxidized under free radical oxidative stress more efficiently (Cossins et al., 1998). The ratio of ascorbic acid to dehydroascorbic acid (AsA/DAsA) was 3.0 in full sun and 6.2 under shading in 2002, respectively (Table 2.4). In 2003, the ratio was 4.2 in full sun and 4.5 under shading, respectively (Table 2.5). The higher AsA/DAsA ratio under shading in both years implied that low light intensity could have reduced the occurrence of free radical-associated damage and that high-content ascorbic acid to inhibit the free radical-associated damage was not as necessary as those in full sun. Moreover, the higher content of vitamin C in 2003 than that in 2002 indicated the effect of light on the formation of vitamin C and implied the function of vitamin C as an antioxidant again.

The results in Table 2.6 indicated that shading did not significantly increase the total N content in tea flushes, though there existed this kind of tendency in 2002. Table 2.6 and Table 2.7 indicated that shading could increase the total contents of P and K in tea flushes and the total contents of Mg, Ca, Al and Mn in tea flushes (data not shown), which was consistent with the results that shading increased concentrations of mineral nutrients (Zhao and Oosterhuis, 2000). The study of Duan and Guo (1992) indicated that in tea garden air temperature was decreased 0.5-4.0°C in summer by shading. The absolute content of soil water and relative humidity of the air were increased 17-40% and 2-20%, respectively, because of the lower velocity and less evaporation under shading. Although the total contents of N, P, K in soil were not affected by shading, the available N, P, K in soil increased significantly, the organic matter content was high up to 7.08%, which significantly improved the quality. Moreover, low light intensity can keep tea plants growing at a reduced rate, which indirectly made the tea flushes to be kept tender for a

longer time. So the changes of microclimate under shading were the main reason causing the increased uptake of most elements.

#### 4.2. Effect of shading and nitrogen on enzyme activities of NR, PAL and PPO

The relationship between light and the activities of NR, PAL and PPO and the relationship between light, enzyme activities and the quality of green tea were simply described and shown in Fig. 2.6.

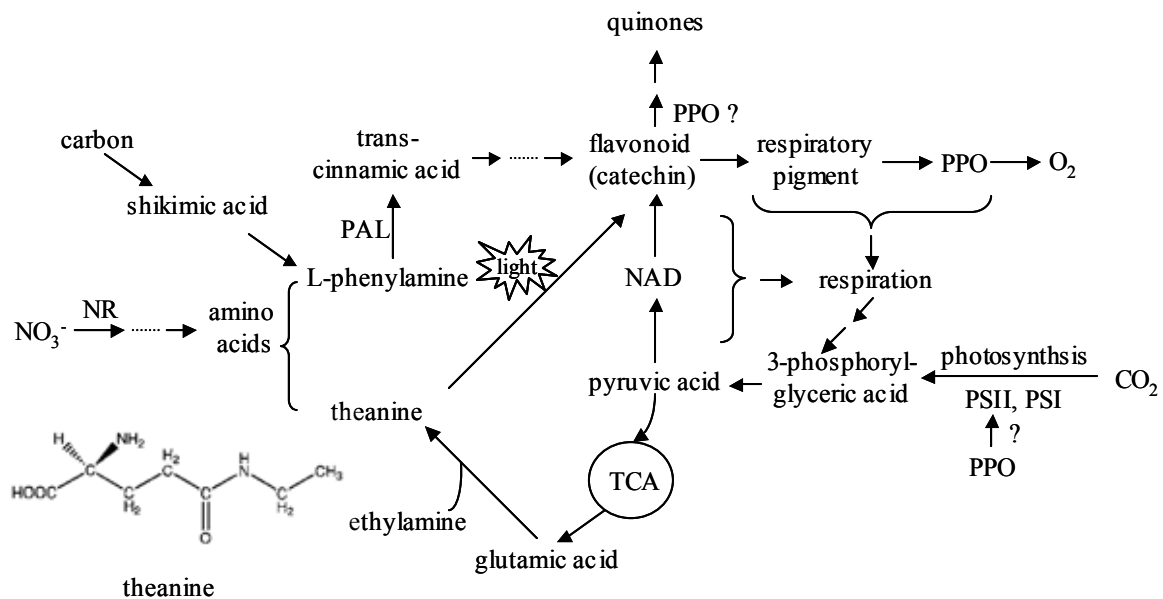


Fig. 2.6 A scheme of effects of light intensity on the activities of nitrate reductase (NR), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) and the relationship between light intensity and enzyme activities as well as the quality of green tea.

Nitrate reductase is considered a key enzyme in the process of assimilation of nitrate in higher plants (Beevers and Hageman, 1969) and is inducible by substrate (Beevers and Hageman, 1969; Ashley et al., 1975) and light (Beevers and Hageman, 1969; Atkins and Canvin, 1975; Nicholas et al., 1976), which promote gene transcription, synthesis of apoprotein and assembly of the holoenzyme (Solomonson and Barber, 1990; Crawford, 1995; Cabello et al., 1998). NR activity could be used for differentiation of metabolically active and inactive N fractions and also for plant N status rather than depending on chemical analysis (Sarmah et al., 1987). Oaks et al. (1988) showed that the induction of

inactive NR protein in corn occurred in response to light in the presence of very low levels of nitrate, while full expression of active enzyme required higher levels of nitrate. Studies in which the kinetics of the appearance of NR protein and the expression of NR activity were followed during “induction” showed that NR protein generally appears more rapidly than NR activity. Ward et al. (1988 and 1989) reported that a latent NR is associated with the plasma membrane of corn roots and barley seedlings. This plasma membrane-bound form of NR may function as a nitrate transporter and accounts for only 1-4% of the total NR activity under normal conditions. Moreover, although the nitrate reductase synthesis is generally repressed in darkness, when the green alga *Monoraphidium braunii* was grown under heterotrophic conditions with glucose as the carbon and energy source, synthesis of NR was maintained in the dark at near-normal levels. NR protein, in fact, appeared to be elevated under these conditions (Diez and Lopez-Ruiz, 1989). In this study, the adopted NR activity is exactly the fully activated enzyme activity and possibly includes the activity of latent plasma membrane-bound form NR. In full sun, higher content of carbohydrate within tea plant was another factor to result in higher NR activity except that light has the inducible effect on NRA (Beevers and Hageman, 1969; Atkins and Canvin, 1975; Nicholas et al., 1976). Therefore, the NR activity in full sun was higher than that under shading. According to this study, the comparison of NR activities in different leaf positions within the year could not be presented, but NR activity in the 1<sup>st</sup> leaf in 2003 was much higher than in 3<sup>rd</sup> leaf in 2002 (Fig 2.2). The NR activity in Fig 2.2 is consistent with the results of Wu and Wu (1993), in which nitrate reductase had the highest activity in the 1<sup>st</sup> leaf. There was no obvious correlation between NR activity and N application rate in soil, though the NR activity can be induced by the substrate of nitrate and nitrate nitrogen in soil had a strongly linear relationship with N application rates (Fig. A9). However, there was a significant effect of light intensity on it. Low light intensity resulted in relative lower NR activity in both-position leaves, because light is an important regulatory signal for NR and NiR expression (Crawford, 1995). Decreased NR activity could partly hinder the synthesis of some aromatic amino acids, such as Phe and Tyr, which are the precursors of cinnamic acid that is the precursor of catechins.

The first committed step for biosynthesis of the phenylpropanoid skeleton in higher plants is the deamination of L-phenylalanine to yield trans-cinnamic acid and ammonia. This reaction is catalyzed by phenylalanine ammonia-lyase and is often regarded as a key step in the biosynthesis of the flavonoids (Lister and Lancaster, 1996). Light has an inducible effect on PAL activity and synthesis or linkage of A ring and hydroxylation of the B ring are promoted by light (Forrest, 1969). The decreased activity of PAL caused by shading verified this finding (Fig. 2.3). Why shading can increase the content of amino acids and decrease the content of tea polyphenols can be understood from the following points: i) The *N*-ethyl carbon of theanine is largely incorporated into the phloroglucinol nucleus of catechins in tea shoots. This incorporation is greatly controlled by light (Kito et al., 1968). These results may well explain the relationship between theanine and catechin content in shaded and exposed tea leaves. ii) Light may be needed for the degradation of theanine in the restricted site of theanine degradation, where the *N*-ethyl carbon of theanine may be converted to the phloroglucinol nucleus of catechins probably by the condensation mechanism via acetyl-CoA (Kito et al., 1968). iii) Less *N*-ethyl carbon is required by the synthesis of catechin caused by low activity of carbon metabolism at low light intensity, which resulted in the accumulation of theanine. v) The growth of tea plants might be inhibited by shading, less theanine is used to its further metabolism. So more free theanine is left. However, according to the results in this study, no definite explanation can be made. So it is still necessary to investigate the physiological basis for this relationship to support any above hypothetical explanation.

Another important enzyme related to polyphenols is polyphenol oxidase (PPO). Plant PPO are located in plastids in healthy plant cells, such as chloroplast, where it is bound to the thylakoid membranes, although reports of their occurrence in other cell compartments are quite abundant (Mayer and Harel, 1979; Mayer, 1987). The physiological function of the enzyme in plants is still obscure after a century of intensive research. Various roles have been postulated for it, such as, an enzyme playing a role in host defense mechanisms, or as a mediator of photosynthetic electron transport. Universally, PPO is thought to oxidize a variety of phenolic substrates, including tyrosine, to L-dihydroxyphenylalanine and then to quinones. Normally PPO is considered to be an essential enzyme during black tea

manufacture (withering, rolling, fermentation and drying) (Latha and Ramarethinam, 2000; Ravichandran and Parthiban, 1998; Liyanage et al., 1997), in that PPO activity decreased during fermentation and pre-firing phases of processing and increases in PPO activities were observed during rolling stage. In most of the cases, PPO is thought to react directly with phenolic substrates that come out of the vacuole after the cells are broken while the leaves are being rolled. In contrast, studies of PPO in green tea are relatively rare, only limited information about PPO was reported by Ye et al. (1994) in the study of the relationship between sulphur and quality of green tea, which demonstrated that sulphur inhibited the activity of PPO. But it was simply argued that the decreased PPO activity hindered the metabolism of carbon, which inhibited synthesis of polyphenols. This study also indicated the shading could significantly increase the activity of PPO in tea flushes (Fig. 2.3). While the function of PPO in the chloroplast remains unresolved, the finding of PPO in PS II particles increased the need to assess any role that PPO may play in the electron transport of that photosystem, whether structural or functional (Hind et al., 1995). Thus, the increased PPO activity in shaded growth condition could not be merely and simply linked to the decreasing of tea polyphenols in tea flushes, its role in biological oxidation within tea plants also should be taken into consideration, because it is thought to function at the step from respiratory pigment oxidized to O<sub>2</sub> in situ. As for the physiological function of PPO in green tea, it still needs much further work on it. In any case, the combination of these three enzymes could provide some threads to explain why shading can improve the quality of tea, though the explanations are not overall.

#### **4.3. Effect of shading and nitrogen on photosynthesis**

Plants are capable of adaptation to their light environmental conditions (Berry, 1975). But the range of the adaptability varies for each plant. Under shading condition, leaves became thinner and larger, resulting in a higher specific leaf area. Similar morphological responses were noted for other tropical fruit trees such as carambola (Marler et al., 1994) and mangosteen (Wiebel et al., 1994). This modification to thinner and larger leaves is a common adaptation to low irradiance (Higuchi et al., 2001) and might be looked at as a strategy to maximize light interception, that is, to maximize the net rate of energy capture



(Givnish, 1988). The ability to accommodate shading through morphological adjustment is associated with a relatively high investment in leafy exploration shoots (Dupré et al., 1986 in Hees, 1997). This growth pattern seems to maximize light capture with a restricted investment in woody biomass. Leaf chlorophyll content on the basis of unit leaf area generally indicated a tendency of an increase with shading. The increase of chlorophyll content and the decrease of chlorophyll a/b (Table 2.8 and Table 2.9) were consistent with those reported for pea (Anderson, 1986), kiwifruit (Chartzoulakis et al., 1993) and mango (Schaffer and Gaye, 1989). However, SPAD values under shading were a little bit lower than those in full sun (Table 2.10), which was possibly caused by the error of taking samples and could not overturn the fact that shading can cause the increase of chlorophyll. This increasing tendency of chlorophyll content might also be an acclimation to low light environment to enhance the light use efficiency. The increase in chlorophyll concentration, especially chlorophyll b of shaded leaves might be associated with the adaptation of leaves to low light environment (Barker, 1985 in Zhao and Oosterhuis, 1998) to intercept more light energy, or with a decreased chlorophyll degradation rate (Hale and Orcutt, 1987 in Zhao and Oosterhuis, 1998). A shaded environment obviously has a relatively low irradiance, but this is usually associated with an altered spectral distribution of light. The spectral quality is altered primarily by light absorption of chlorophyll in the leaf canopy. Shadelight, by which is meant the light within leaf canopies such as at the ground level in dense forests, is different from sunlight in that it is relatively deficient in red light (600-680 nm) while being enriched in wavelengths greater than 700 nm (Evans, 1988). In this study, the change of spectral quality caused by shading net might be less than those resulted from the self-shading of leaves. But the results of Kittas et al. (1999) indicated that covering the greenhouse with shading net at least could slightly decrease the ratio of red/far red in it. The decreasing of red light is not beneficial to the formation of chlorophyll a. Increasing the chlorophyll b content may also increase the proportion of blue light captured by photosystem II (Anderson, 1982). The blue light captured has considerable effects on promoting the N metabolism and its related biochemical reactions to increase the content of N compounds and then to improve the quality of green tea. It has also been suggested that shadelight is

more effective in exciting photosystem I than sunlight and that, to redress this imbalance, shaded plants have a larger ratio of photosystem II to photosystem I, in terms of both chlorophyll or reaction centers (Björkman, 1981 in Evans, 1988). Meanwhile light-harvesting chlorophyll a/b protein complex (LHC) that captures and transmits the light energy in photosystem locates in photosystem II. The plant grown in natural shade has more LHC and the proportion of chlorophyll associated with LHC was increased. So the content of chlorophyll b was increased with the relative increasing of LHC. Moreover, the relative increase in chlorophyll b may enhance the ability of shaded leaves to capture and use the light containing wavelengths that are enriched in the infra-red region of the spectrum (Chartzoulakis et al., 1993).

High light intensity decreased  $g_s$ ,  $C_i$  and  $E$  again (Table 2.10), which are consistent with the reported results of fruit trees (Higgins et al., 1992). Shading was effective to decrease the leaf temperature, easing of water stress. The effect of shading on increasing  $g_s$  may be attributed to increase  $P_n$ , compensating for the effect of low light intensity (Higuchi et al., 2001). In this study, only in the treatment of  $600 \text{ kg N ha}^{-1} \text{ a}^{-1}$ , the results of  $P_n$  within the range of 100-1500 PPFD followed the rule, the results in control completely did not follow it (Fig 2.4). Moreover,  $A_{\max}$  and  $I_c$  in both N levels were all lower in low light intensity than those in full sun (Table 2.10). So the fact that  $P_n$  including the  $A_{\max}$  and  $I_c$  as well as  $R_d$  and leaf nitrogen content per unit leaf area were all lower in shaded leaves (Fig. 2.4 and Table 2.10) suggested that the light reaction in shaded leaves was less efficient than that in exposed leaves, though generally the chlorophyll content was increased and leaf morphology or chloroplast structure were more suitable to capture light energy. This is one of the reasons why shading reduced the yield. Combination with enzyme activities, we can initially explain why shading can reduce the yield at the same time of improving the quality.

#### **4.4. Effect of shading and nitrogen on plant antioxidant power**

Recently, radical scavengers have attracted special interest because they can protect human body from the damage of free radicals that may cause many diseases including cancer and lead to the aging process (Sorata et al., 1984; Nakayama et al., 1993). The

study of Benzie and Szeto (1999) has confirmed that the antioxidant power of green tea is considerably higher than black tea, while Oolong tea, expected to be intermediate, appeared similar to black tea. The potential for all types of tea to contribute significantly to the dietary intake of antioxidant power is high, as one cup of black tea (1-2%) contains around 800  $\mu\text{mol}$  of antioxidant power and several cups of green tea offer the same antioxidant potential as almost 1 g of pure vitamin C. Vitamin C is an antioxidant of established importance, while the bioavailability and role of tea antioxidants are not yet clear, though the ability of ascorbic acid to act as a pro-oxidant is well documented by Laudicina and Marnett (1990). However, the study of Yen et al. (1997) indicated that the pro-oxidant effect was only shown at additional lower dosage but not the effect of natural antioxidants in plants. The study of Benzie and Szeto (1999) indicated that the antioxidant power of all tea correlated strongly with total phenolics content. But the observations of Sawai and Moon (2000) suggested that ascorbic acid scavenges radicals faster and has stronger reducing activity than ethyl gallate.

It is well documented that light energy harnessed by chlorophyll, which cannot be dissipated via photosynthesis on time, can lead to the formation of oxygen free radicals, especially under water-limiting conditions (Kaiser, 1987; Larson, 1988; Navari-Izzo, et al., 1997). The investigation into the role of light during desiccation (Farrant et al., 2003) suggests that APX and GR activity increased only in plants dried in the light. Plants dried in the dark had no measurable activity. The lack of increased antioxidant activity in plants could have allowed for accrual of free radical-associated damage, which in turn contributed to their death. Our study also agreed with these results in that the activities of APX, DHAR and GR activity in full sun under the high irradiance and drought conditions were all slightly higher than those under shading (Fig 2.5), which implied that for the shaded leaves, a light driven regulation of the antioxidant and anthocyanin protection system may not be as vital as that in full sun. So it can be at least partly used to explain why the contents of ascorbic acid in tea flushes (Table 2.4 and Table 2.5) and in mature leaves (Table 2.11) under shading were lower than that in full sun. It also indicated that shading could reduce the risk of free radical damage for tea plants.

This study only presented a few possible explanations how light intensity affects yield and quality of green tea. But light is almost involved in all the metabolisms within tea plant, the explanations in this study are limited and more work related to this is still needed. Moreover, the physiological function of PPO in the metabolism of polyphenols, photosynthesis (PSII) and respiration of green tea should be give more consideration in future work.

## Chapter 3

**Yield and quality of green tea (*Camellia sinensis* L.) as influenced by N fertilizer and light intensity – possibility to estimate optimum N fertilizer demand****Abstract**

Seven-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Longjing43) in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences (30°16' N and 120°12' E) were used for the study of N nutrient diagnosis. N field experiment was a 6×4 factorial arranged in a completely randomized block design with 6 N levels (0, 150, 300, 600, 900, 1600 kg N ha<sup>-1</sup> a<sup>-1</sup>) and four replications and it was carried out on Red Soil in 2002 and 2003. The effect of shading on N nutrient status diagnosis was studied in summer with 2 light intensities (100% of sun and 36% of sun) as well. Only in 2003, the yield showed a significant quadratic polynomial regression with N application rates. 810 kg N ha<sup>-1</sup> a<sup>-1</sup> of the optimum N application rate (N<sub>opt</sub>) calculated from 95% of the maximum yield was regarded more rational than that from 90% of the value, because it was closer to that to reach the lowest TP/AA (the best quality). Shading decreased the N<sub>opt</sub> to 700 kg N ha<sup>-1</sup> a<sup>-1</sup>. Soil N<sub>min</sub> in spring, autumn and summer could index the total yield and quality, but soil N<sub>min</sub> could not index the differences in yield and quality under shading and in full sun. Compared to soil N<sub>min</sub>, plant analysis was a faster and more sensitive method, especially under shading condition. Moreover, NO<sub>3</sub><sup>-</sup> measured with nitrate test strips (RQflex) and amino acid nitrogen (AAN) in young stem, new fully expanded leaves (NFEL) and top old leaves (TOL) in spring, NO<sub>3</sub><sup>-</sup> and AAN in mature leaves (ML) in autumn and NO<sub>3</sub><sup>-</sup> in young stem under shading and in full sun in summer all could reflect soil N<sub>min</sub>, N application rate and index the total yield and quality. In spring, the critical contents of plant indicators were 30-33 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> in the young stem, 6-7 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> in NFEL, 10-11 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> in TOL and 714-732 mg L<sup>-1</sup> AAN in NFEL, respectively. In autumn, they were 5-6 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> and 495-510 mg L<sup>-1</sup> AAN in ML,

respectively. In summer, they were 32-51 mg L<sup>-1</sup> under shading and 20-33 mg L<sup>-1</sup> in full sun. The critical soil N<sub>min</sub> values in 0-60 cm soil depth were 200-260 kg N ha<sup>-1</sup> and 320-430 kg N ha<sup>-1</sup> in spring and in autumn, respectively. 240-340 kg N ha<sup>-1</sup> and 130-230 kg N ha<sup>-1</sup> of optimum N application rate (N<sub>opts</sub>) were obtained in spring and in autumn, respectively. Shading had no effect on critical soil N<sub>min</sub> (180-230 kg N ha<sup>-1</sup> in both shaded and exposed conditions) and the optimum N application rate for summer tea (140-240 kg N ha<sup>-1</sup> in both shaded and exposed conditions). According to this study, N application rate in autumn could be reduced and applied to spring tea. If the measure of shading is applied and summer tea is plucked to make green tea, the N application rate for summer tea can be increased correspondingly.

## 1. Introduction

Modern production of tea requires efficient, sustainable, and environmentally sound management practices. Nitrogen is normally a key factor in achieving optimum green tea yields. Selection of the most appropriate rate of N fertilizer is one of the management practices that can affect both economic viability of tea production and impact of agriculture on the environment.

Large doses of nitrogen fertilizer are usually applied in tea cultivation, because the quality of green tea is markedly correlated with the contents of total nitrogen and total amino acids, which is a major factor determining the freshness and mellowness of green tea and is positively correlated with the quality of green tea (Wang et al., 1988). Therefore, tea growers have applied increasingly larger amounts of nitrogen fertilizers in tea fields. In Japan, the amount of N applied can reach 1200 kg N ha<sup>-1</sup> a<sup>-1</sup> (Watanabe, 1995). But the study indicated that the content of total amino acids in tea flushes (one bud and one leaf) was about 4% with application of 3000 kg N ha<sup>-1</sup> a<sup>-1</sup>, and 3% with 1000 kg N ha<sup>-1</sup> a<sup>-1</sup> (Watanabe, 1995). These results indicated that nitrogen fertilizer was not used efficiently. From an environmental perspective, overuse of chemical N fertilizer has been associated with increased levels of nitrate-nitrogen in ground and surface water (Blackmer, 1987 in Krusekopf et al., 2002). Further, proper timing in combination with adequate rate of N

application is crucial to minimize N losses and improve N use efficiency (De Datta and Buresh, 1989). Therefore, there is a great need to develop a better system of fertilizer application to minimize N losses and improve N use efficiency while preserving the present green tea quality and yield.

At present, the usual methods for rapid nutrient diagnosis include SPAD measurement and nitrate measurement. For the former, the chlorophyll meter instantly measures the amount of chlorophyll content, a key indicator of plant health, just simply by clamping the meter over leaf tissue, and receiving an indexed chlorophyll content reading (0-99.9) in less than 2 seconds. For the latter, the equipment of nitrate electrode and reflectrometer are usually taken. Nitrate electrode measures the nitrate content in aqueous solutions simply, accurately and economically. Nitrate reflectrometer (RQflex, test strips) is a small reflection-type photometer (RQflex) to dip the nitrate test strip into the test solution and place it into RQflex to quantify the intensity of the red coloured reaction product from  $\text{NO}_2^-$  formed after the reduction of  $\text{NO}_3^-$  with other reagents.

The first step to build up the system for recommending fertilization is to find the suitable diagnosis indicator. Normally, soil  $N_{\min}$  and plant analysis were all regarded as good indicators (Daugaard and Todsén, 1999), especially plant analysis, which was regarded as a faster and more sensitive method (Lacroix and Cousin, 1997). So far, several plant indicators have been used to discuss plant N status:  $\text{NO}_3^-$  content of the petiole of potatoes (Carl et al., 1996), the base stem of spring wheat (Li et al., 1997) and the N content of the entire aboveground in wheat (Baethgen and Alley, 1989). However, in tea, the situation is most complex, since users are dealing with a perennial plant and the flushes are harvested in rationally intervals. In order to increase yield, tea is plucked not only in spring, but also in autumn. Such intensive plucking causes the tremendous increase of N requirement, thus results in the increase of N application rate. Because of the high cost of fertilizers, assessment of the optimal nitrogen rate required for each crop is essential. To ensure the judicious use of fertilizer, there is therefore a current need for a study of usefulness of organs as indicators of N status of plants and as a predictor of fertilizer used. But so far, little research in this respect has been done.

Due to the influence of shading on the growth and N metabolism in tea plant, the indicators of diagnosis must be different from those in full sun. Therefore, the effect of shading on the plant diagnosis and critical N levels in soil in an addition to the results of N diagnosis in spring and autumn tea will be discussed and supplies additional information to establish the diagnosis model in tea plant.

The objectives of this research were (i) to compare the different measurement methods and to find out the suitable indicators; (ii) to determine the critical indicator contents in spring, summer and autumn; and (iii) to establish the basis of a system for predicting optimum rates of N fertilization for green tea to be used within the same growing season.

## **2. Materials and Methods**

N diagnosis was studied in the N experiment and shading experiment, therefore the experimental designs were identical to Chapter 1 and Chapter 2.

### **2.1. Yield determination**

For N experiment, total fresh yield determination is identical to Chapter 1. For shading, total fresh yield under shading and in full sun was the fresh yield in spring and autumn added to that under shading and in full sun after shading 15 days as described in Chapter 2, respectively. Any difference in yield was caused by shading.

### **2.2. Quality constituents in tea flushes**

The determination of quality constituents is identical to Chapter 1.

### **2.3. Methods to estimate N fertilizer demand**

Chlorophyll is a key indicator of plant health, SPAD meter was used to measure the content of chlorophyll. Tea plant is not as sappy as tomato, potato and spinach and only has the very short petiole, so the petiole is not the ideal organ for diagnosis. Its fiberblast-like stems make the basal stem less suitable as the organ for diagnosis in tea plant. So the  $\text{NO}_3^-$  in young stem and in leaves was taken as studied targets.  $\text{NO}_3^-$  was



determined by nitrate electrode and  $\text{NO}_3^-$  test strips. Amino acid nitrogen (AAN), a promising indicator, which had been used in winter wheat by Schulz and Marschner (1987), was also considered in this study because of its importance to the quality of green tea. AAN was measured with ninhydrin method based on the standard of L-glutamic acid (Tea Research Institute, 1983).

#### **2.4. Soil and plant samples collection**

0-30 cm and 30-60 cm soil samples were taken with soil auger ("Pürckhauer"), transported to the lab in an ice box and frozen in the deepfreeze. Soil  $N_{\min}$  was measured by the method of Wehrmann and Scharp (1986).  $1.264 \times 10^3 \text{ kg m}^{-3}$  of soil density according to Peng et al. (2003) was taken in the calculation of soil  $N_{\min}$ . Tea plant samples consisting of the young stem, new fully expanded leaves (NFEL) and top old leaves (TOL, which were on the brown stems and grew up from the former year) in spring, the young stem and mature leaves (ML) in autumn (because it is difficult to differentiate between the new fully expanded leaves and the completely mature leaves) and the young stem and mature leaves in summer were taken at the same time when taking  $N_{\min}$  soil samples. In spring, soil samples were taken on April 22, 50 days later after N fertilization; in autumn, soil samples were taken on September 20, 29 days later after N fertilization; in summer, soil samples were taken on August 23, 81 days later after N fertilization. Plant sampling was finished before 11 o'clock in the morning. The plant samples were taken to the lab and the pressed sap immediately obtained for the analysis of nitrate and amino acid nitrogen.

The pressed sap can be directly used to the measurement of  $\text{NO}_3^-$  without dilution. However, the pressed sap had to be diluted by 40 and 20 for the determination of the content of amino acid nitrogen in leaves and stem, respectively.

#### **2.5. Data analysis of diagnosis**

According to the definition that critical or sufficient levels are the plant tissue N concentration ( $\text{g kg}^{-1}$ ) that produced 90% of the maximum yield (Baethgen and Alley, 1989), we can define the critical or sufficient levels as indicator content ( $\text{NO}_3^-$  and AAN,

mg L<sup>-1</sup>) in the tea plant tissues that produced 90% of the maximum yield. But in the practices of production, the nutrient content at the yield that was 5-10% below the maximum yield was defined as the critical level (Li et al., 1997). Moreover, green tea is a cash crop and its quality is essential to green tea. Therefore, 95% of the maximum yield and the lowest TP/AA ratio were also considered when the critical indicator contents were calculated.

In most practices of production, quadratic polynomial was taken. So the responses of the yield of green tea to different N application rates and indicators were studied using nonlinear quadratic polynomial regression analysis in three seasons. Because of the importance of tea quality, the response of tea quality (TP/AA) to different indicators was also studied using quadratic polynomial function. The maximum yield value for the given response curve is the yield value that makes the first derivative of the response curve function equal to zero. The soil N<sub>min</sub> and indicator contents (NO<sub>3</sub><sup>-</sup> and AAN) in the tissues at 90% and 95% of the maximum yield can be calculated from the maximum yield obtained from the given curve function. The best quality value for any given response curve is the TP/AA ratio that makes the first derivative of the response function equal to zero. So soil N<sub>min</sub>, NO<sub>3</sub><sup>-</sup> and AAN contents at the best quality in the tissues can be calculated.

All the critical NO<sub>3</sub><sup>-</sup> and AAN content values were fitted back to the linear functions between plant indicator content (NO<sub>3</sub><sup>-</sup> and AAN) and soil N<sub>min</sub> to calculate the critical soil N<sub>min</sub> in three seasons. Each critical soil N<sub>min</sub> was fitted back to the linear function between soil N<sub>min</sub> and N application rate in spring, autumn and summer to obtain the critical or the optimum N application rate within the season, respectively.

### **3. Results**

#### **3.1. Yield and the optimum N application rate**

The total fresh yields of both years did not show evident response to nitrogen application rates, and although the yield in 2003 showed a stronger response to different N levels, the differences were still not significant (Fig. 3.1). However, significant differences between

years were found. Compared to 2002, the yield responses to N application rates in 2003 were more significant. Applying a quadratic polynomial function to describe the relationship between yield and N application rate, the optimum N application rate (90% of the maximum yield) could be deduced,  $-370 \text{ kg N ha}^{-1} \text{ a}^{-1}$  (2002) and  $460 \text{ kg N ha}^{-1} \text{ a}^{-1}$  (2003). If not 90%, but 95% of the maximum yield was considered, the optimal N application rate increased significantly, i. e.,  $380 \text{ kg N ha}^{-1} \text{ a}^{-1}$  (2002) and  $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  (2003).

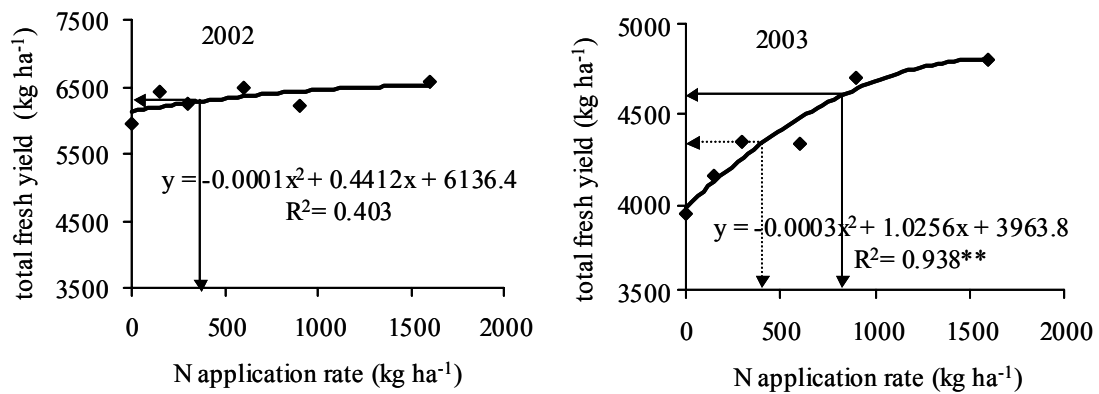


Fig. 3.1 The yield of green tea as influenced by the nitrogen fertilization (the broken line for 90% of the maximum yield and solid line for 95% of the maximum yield)

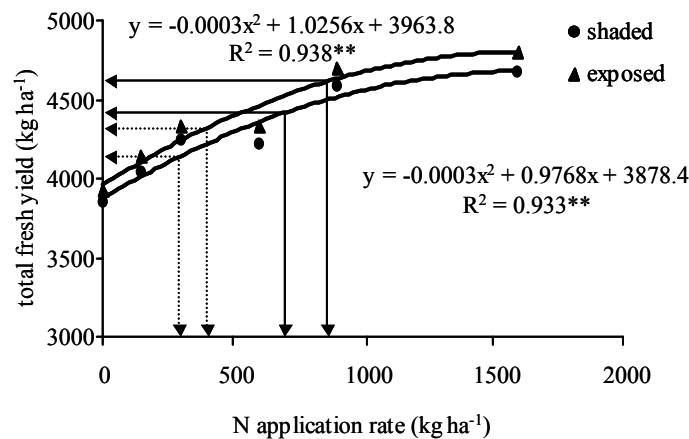


Fig. 3.2 The total fresh yield of green tea as influenced by shading and the nitrogen levels in 2003 (the broken line for 90% of the maximum yield and the solid line for 95% of the maximum yield).

Because of the small yield response to N application rates in 2002 (Fig. 3.1), only the results for shading in 2003 was presented. The total fresh yield under shaded and exposed growth conditions were significantly nonlinearly correlated with nitrogen application rates

(Fig. 3.2 exposed:  $R^2=0.938^{**}$ ; shaded:  $R^2=0.933^{**}$ ), which followed a quadratic polynomial function (Fig. 3.2). The optimum N application rate at 90% of the maximum yield were 440 kg N ha<sup>-1</sup> a<sup>-1</sup> in full sun and 350 kg N ha<sup>-1</sup> a<sup>-1</sup> under shading. If 95% of the maximum yield was used, the optimum N application rates ( $N_{\text{opty}}$ ) were 810 kg N ha<sup>-1</sup> a<sup>-1</sup> and 700 kg N ha<sup>-1</sup> a<sup>-1</sup>, respectively.

Due to the high price for tea (55 RMB kg<sup>-1</sup>), the cost for additional fertilizer to receive 95% of the maximum yield was profitable, as was shown in Table 3.1.

Table 3.1 The effect of the calculation of the optimum yield between 90% of the maximum yield (90% $Y_{\text{max}}$ , FW kg ha<sup>-1</sup> a<sup>-1</sup>) and 95% of the maximum yield (95% $Y_{\text{max}}$ , FW kg ha<sup>-1</sup> a<sup>-1</sup>) on the profit of farmers (Profit, RMB ha<sup>-1</sup> a<sup>-1</sup>) from the difference of the output value from made tea and expenditure for fertilizer. ( $\Delta Y$ : 95% $Y_{\text{max}}$  - 90% $Y_{\text{max}}$ , kg ha<sup>-1</sup> a<sup>-1</sup>;  $\Delta N$ : the difference of N applied rates at each yield level, (kg ha<sup>-1</sup> a<sup>-1</sup>);  $\Delta$ output value: difference between output values of 95% $Y_{\text{max}}$  and 90%  $Y_{\text{max}}$ , (RMB ha<sup>-1</sup> a<sup>-1</sup>);  $\Delta$ expenditure: difference between expenditures of 95% $Y_{\text{max}}$  and 90%  $Y_{\text{max}}$ , (RMB ha<sup>-1</sup> a<sup>-1</sup>)).

	90% $Y_{\text{max}}$	95% $Y_{\text{max}}$	$\Delta Y$	$\Delta N$	$\Delta$ output value from tea	$\Delta$ expenditure from fertilizer	Profit
Yield	4356	4598	242		3328*		
N fertilizer	439	811		372		1013 <sup>‡</sup>	2315

\*: The output value was calculated from made tea, normally 1 kg made tea is made from 4 kg fresh tea (the ratio from TRI in China). The price of made tea is 55 RMB kg<sup>-1</sup> (average price from 1996 to 2000, data from the Agricultural Provincial Government Department of Zhejiang Province, personal communication).

<sup>‡</sup>: Fertilizer (urea) price is 1.253 RMB kg<sup>-1</sup> (the export price in September of 2003 from [www.nateesc.gov.cn](http://www.nateesc.gov.cn)).

### 3.2. N removal and N balance

N removal and N balance were shown in Fig. 3.3. In 2002, N removal did not show a significant quadratic polynomial regression with N application rate, and N removal was 70-80 kg N ha<sup>-1</sup> a<sup>-1</sup> by plucking. But in 2003, N removal showed a significant quadratic polynomial regression with N application rates and N removal was 40-55 kg N ha<sup>-1</sup> a<sup>-1</sup>.

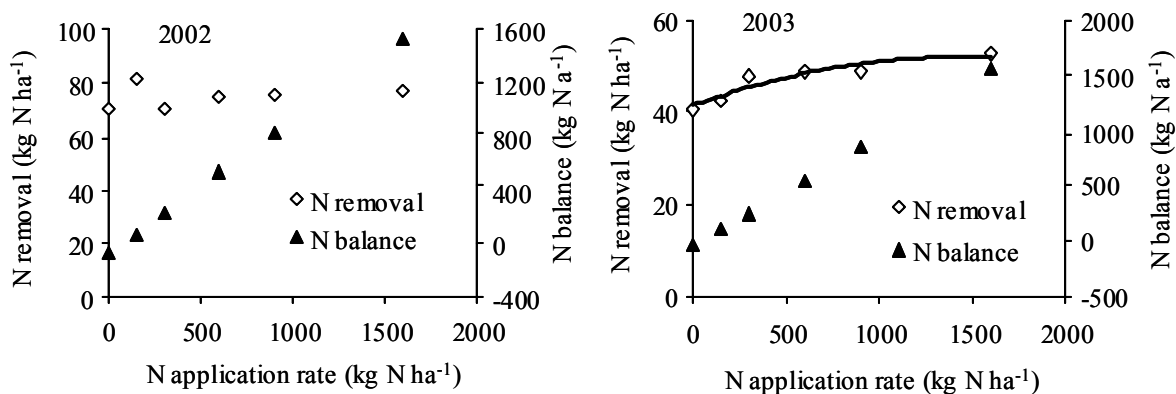


Fig. 3.3 N removal by plucking and N balance in tea production system in 2002 and 2003.

### 3.3. Diagnosis of N status based on yield and quality

Because of the small yield response to N application rate in 2002 (Fig. 3.1) and not significant response of N removal to N application rate in 2002 (Fig. 3.3), only the results related to diagnosis in 2003 were presented in this study.

#### 3.3.1. Comparison of methods to estimate N fertilizer demand

SPAD measurements (SPAD-502) are easily performed, however, although SPAD value can represent the total content of chlorophyll (Fig. 3.4 A,  $R^2=0.938^{**}$ ), it was poorly correlated with N application rate, even negative correlation (Fig. 3.4 B,  $R^2=0.138$ ), which indicated the chlorophyll could not respond to the nitrogen supplied, so the chlorophyll meter can not be used in the rapid test of nutrient diagnosis in tea plant.

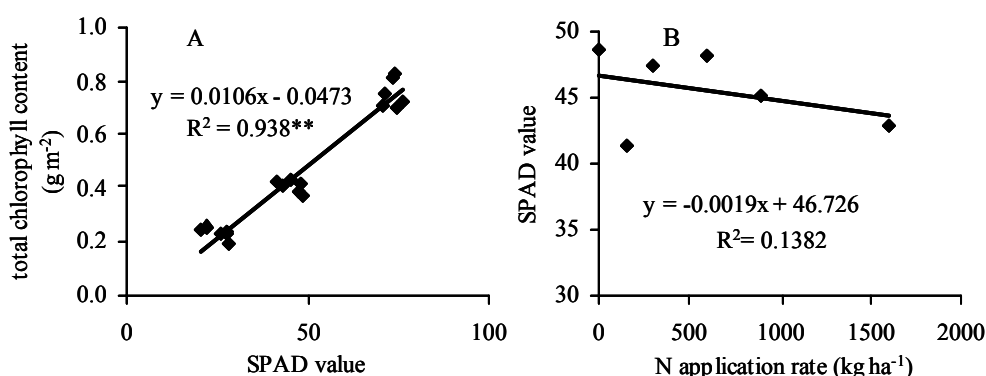


Fig. 3.4 The relationship between total chlorophyll, SPAD values of tea flushes, new fully expanded leaves and mature leaves (A,  $df=18$ ) and the relationship between SPAD values of new fully expanded leaves and N application rates (B,  $df=6$ ).

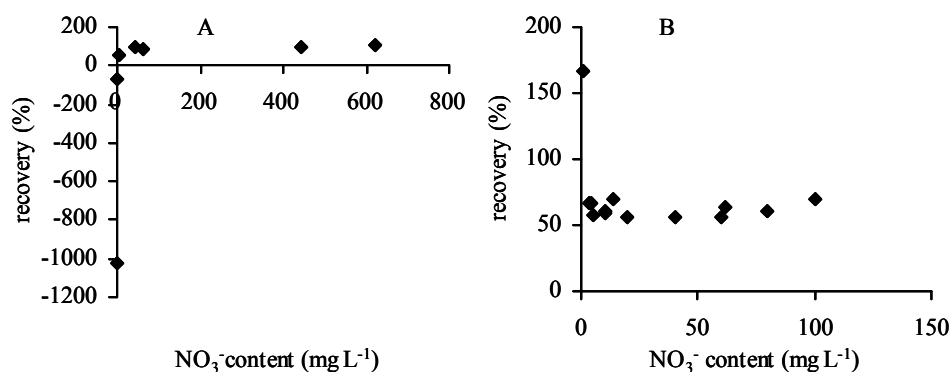


Fig. 3.5 The effect of leaf sap on the recovery of nitrate measured with nitrate electrode (A) and reflectrometer (B). Average value is the mean of 3 replications.

In order to compare the accuracy of the methods using nitrate electrode (Thermo Orion, Model 9707) and reflectrometer (test strips) (Merck, RQflex 2) to measure  $\text{NO}_3^-$ , the standard addition technique by an adaptation of Rosen et al. (1996) was used. The new fully expanded and mature leaves were taken in 6 N-level treatments into one large sample and homogenized. The filtered solution was used as the adding sap. Standard addition of  $\text{NO}_3^-$  at 1, 2, 3, 4, 5, 10, 20, 40, 60, 80  $\text{mg L}^{-1}$  sap were made by adding the required amount of high-concentration potassium nitrate solution (1000  $\text{mg L}^{-1}$   $\text{KNO}_3$  for 20-80  $\text{mg L}^{-1}$   $\text{NO}_3^-$ -sap solution, 100  $\text{mg L}^{-1}$   $\text{KNO}_3$  for 2-10  $\text{mg L}^{-1}$   $\text{NO}_3^-$ -sap solution and 10  $\text{mg L}^{-1}$   $\text{KNO}_3$  for 1  $\text{mg L}^{-1}$   $\text{NO}_3^-$ -sap solution) in a 25-ml volumetric flask and adding sap to bring up to volume. All  $\text{NO}_3^-$  determinations were made in triplicate using nitrate electrode (0.5 ml 26%  $\text{Al}_2(\text{SO}_4)_3$  was added as the interference suppressor solution) and reflectrometer (teat strips). The results of recovery are shown in Fig. 3.5, which indicated that the sensitivity of nitrate electrode was easily affected by the leaf sap, especially in the lower standard  $\text{NO}_3^-$  concentration ( $\text{NO}_3^- < 1 \text{ mg L}^{-1}$ ). But in the higher  $\text{NO}_3^-$  standard concentration ( $\text{NO}_3^- > 630 \text{ mg L}^{-1}$ ), the electrode was also not stable, despite of the good accuracy with a recovery of 97.2% for  $\text{NO}_3^-$  in pure distilled water (data included in the Fig. 3.5 A). In contrast, although the accuracy of reflectrometer was not as good as that of nitrate electrode and its recovery of  $\text{NO}_3^-$  was only 68% in pure distilled water, the general recovery was quite stable except when  $\text{NO}_3^-$  concentration was as low as 1  $\text{mg L}^{-1}$  (Fig. 3.5 B). But in most cases, the  $\text{NO}_3^-$  content in tea leaves is higher than 1  $\text{mg L}^{-1}$ , which

meant that sample matrix had less effect on the measurement of  $\text{NO}_3^-$  generally. Compared to reflectrometer, nitrate electrode is too easily affected by sample matrix or sensitive to the interferential ions as well as easily affected by water quality, all of which indicate that nitrate electrode is not suitable to the rapid test of nutrient diagnosis in tea plant, especially in the field. It is evident that reflectrometer is the best choice among them. The benefits of using RQflex 2 system include: i) portable device allowing on site use; ii) easy to handle system; iii) reliable results within minutes; v) application notes with each kit; vi) low cost analysis; vii) a wide range of analyses available.

The relationship between  $\text{NO}_3^-$  and AAN in new fully expanded leaves in the spring of 2003 was shown in Fig. 3.6, which indicated that  $\text{NO}_3^-$  was significantly correlated with AAN in the same organ and implied that  $\text{NO}_3^-$  measurement using RQflex was accurate.

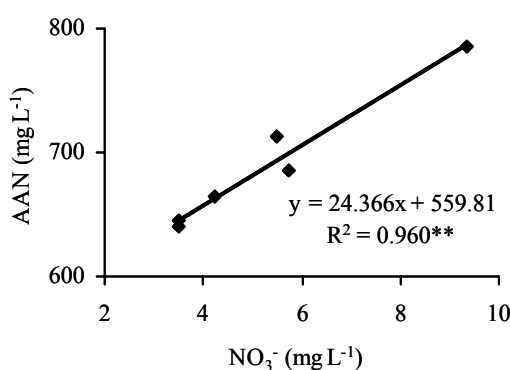


Fig. 3.6 The relationship between  $\text{NO}_3^-$  content and amino acid nitrogen (AAN) content in the pressed sap of new fully expanded leaves in the spring of 2003.

### 3.3.2. Possible indicators for N fertilizer demand recommendation

#### 3.3.2.1. Soil $N_{\min}$ in 0-60 cm soil depth

Soil  $N_{\min}$  in spring and autumn could index not only the total yield but also the quality (TP/AA). A quadratic polynomial function could reflect the responses of yield and quality to soil  $N_{\min}$  (Fig. 3.7). In spring, the optimum soil  $N_{\min}$  values at 90%, 95% of the maximum yield and the lowest TP/AA (best quality) were 130, 200 and 220 kg N ha<sup>-1</sup>, respectively; in autumn, they were 270, 360 and 350 kg N ha<sup>-1</sup>, respectively. Compared to

spring, the indexed effect of soil  $N_{\min}$  to yield in autumn was not as good as that in spring, but soil  $N_{\min}$  in autumn could index the quality better than that in spring. In summer, the responses of yield and quality to soil  $N_{\min}$  (Fig. 3.8) indicated that soil  $N_{\min}$  could significantly index the total yield and quality under shading and in full sun. The optimum soil  $N_{\min}$  values were 195 kg N ha<sup>-1</sup> (90% of the maximum yield), 250 kg N ha<sup>-1</sup> (95% of the maximum yield) and 220 kg N ha<sup>-1</sup> (the lowest TP/AA) under shading, respectively. In full sun, they were 190, 250 and 230 kg N ha<sup>-1</sup>, respectively.

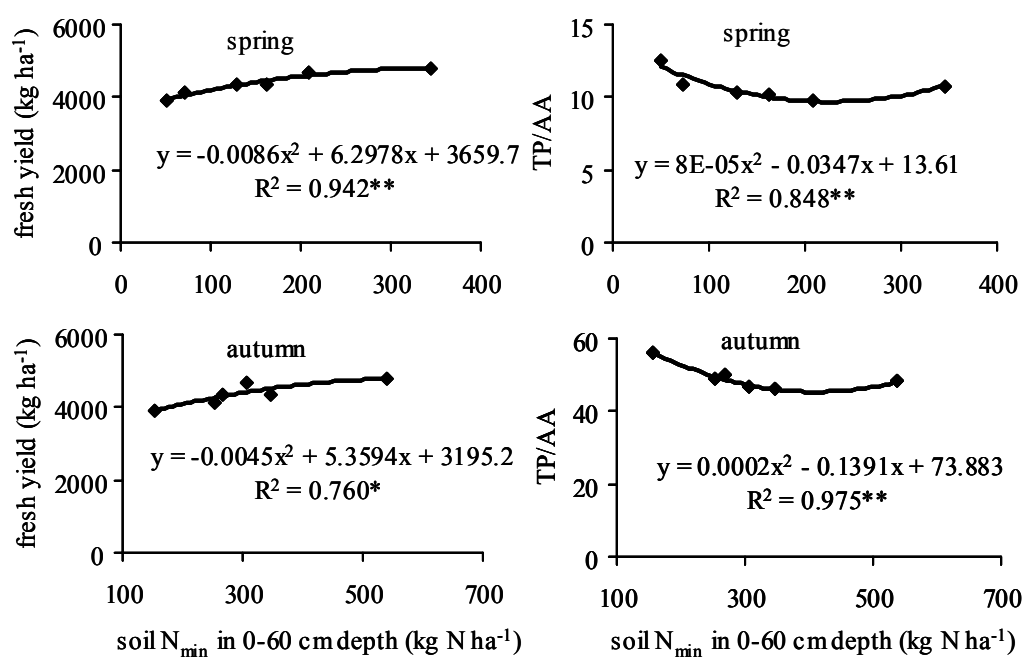


Fig. 3.7 The relationship between soil  $N_{\min}$  in 0-60 cm soil depth and yield as well as quality in spring and autumn of 2003.

Considering the economic reason, the optimum soil  $N_{\min}$  values from 95% of the maximum yield to the lowest quality were regarded as the optimum soil  $N_{\min}$ . Therefore, they were 200-220 kg N ha<sup>-1</sup> in spring, 350-360 kg N ha<sup>-1</sup> and 220-250 kg N ha<sup>-1</sup> under shading and 230-250 kg N ha<sup>-1</sup> in full sun in summer. It was evident that shading had no significant effect on optimum soil  $N_{\min}$  in summer.



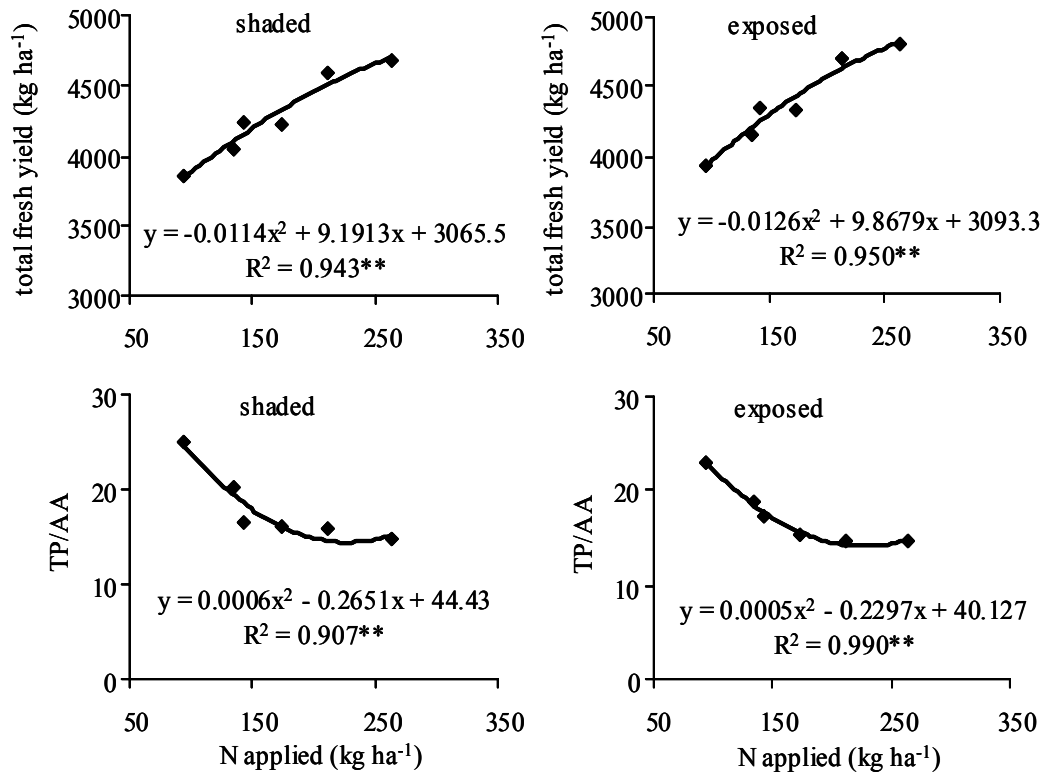


Fig. 3.8 The relationship between soil  $N_{min}$  and total fresh yield as well as quality under shaded and exposed conditions in summer of 2003.

### 3.3.2.2. Total plant N content, $NO_3^-$ and amino acid nitrogen (AAN)

N application rates had no effect on the total content of N in tea flushes in both years and total content of N in spring tea flushes was much higher than that in autumn ones (Fig. 3.9).  $NO_3^-$  and AAN contents in different parts of tea plant in 2003 also indicated that N only had slightly significant effects on the content of free amino acids in flushes, but  $NO_3^-$  in young stem and  $NO_3^-$  and AAN in new fully expanded leaves in spring (Fig. 3.10 A, B) and  $NO_3^-$  in young stem and  $NO_3^-$  and AAN in mature leaves in autumn (Fig. 3.10 C, D) were all strongly and significantly affected by N application rates. The content of free amino acids in spring tea flushes was higher than that in autumn ones.

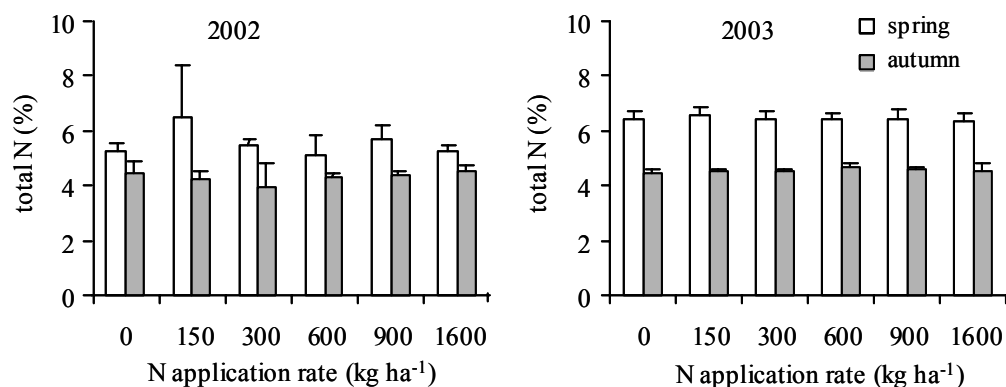


Fig. 3.9 Effects of N on the total content of N in green tea flushes (one bud and one leaf) in both years.

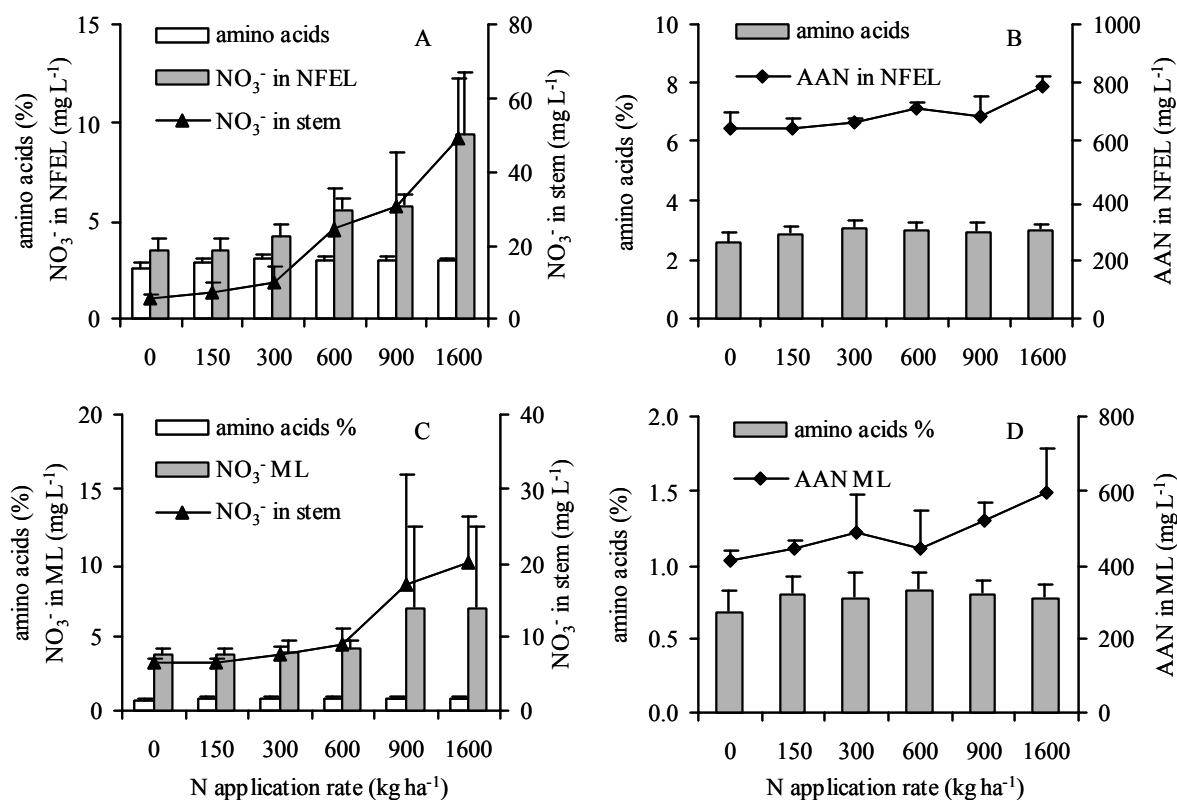


Fig. 3.10 Effects of N levels on the contents of amino acids in flushes (one bud and one leaf) and  $\text{NO}_3^-$  in young stem and amino acids nitrogen (AAN) in new fully expanded leaves (NFEL) in spring (A, B) and  $\text{NO}_3^-$  in young stem and AAN in mature leaves (ML) in autumn (C, D) in 2003.

### 3.3.3. Plant indicators and total fresh yield as well as quality

In spring, total fresh yield was significantly correlated with  $\text{NO}_3^-$  in young stem (Fig. 3.11 A,  $R^2=0.857^{**}$ ),  $\text{NO}_3^-$  in new fully expanded leaves (Fig. 3.11 C,  $R^2=0.843^{**}$ ) and  $\text{NO}_3^-$  in top old leaves (Fig. 3.11 E,  $R^2=0.851^{**}$ ), following a quadratic polynomial function. But total fresh yield was only significantly correlated with AAN in new fully expanded leaves (Fig. 3.11 B,  $R^2=0.697^*$ ). Although the total fresh yield also followed a quadratic polynomial regression with AAN in top old leaves, its regression was converse with the others in Fig. 3.11. It indicated that AAN in top old leaves was not a good indicator in

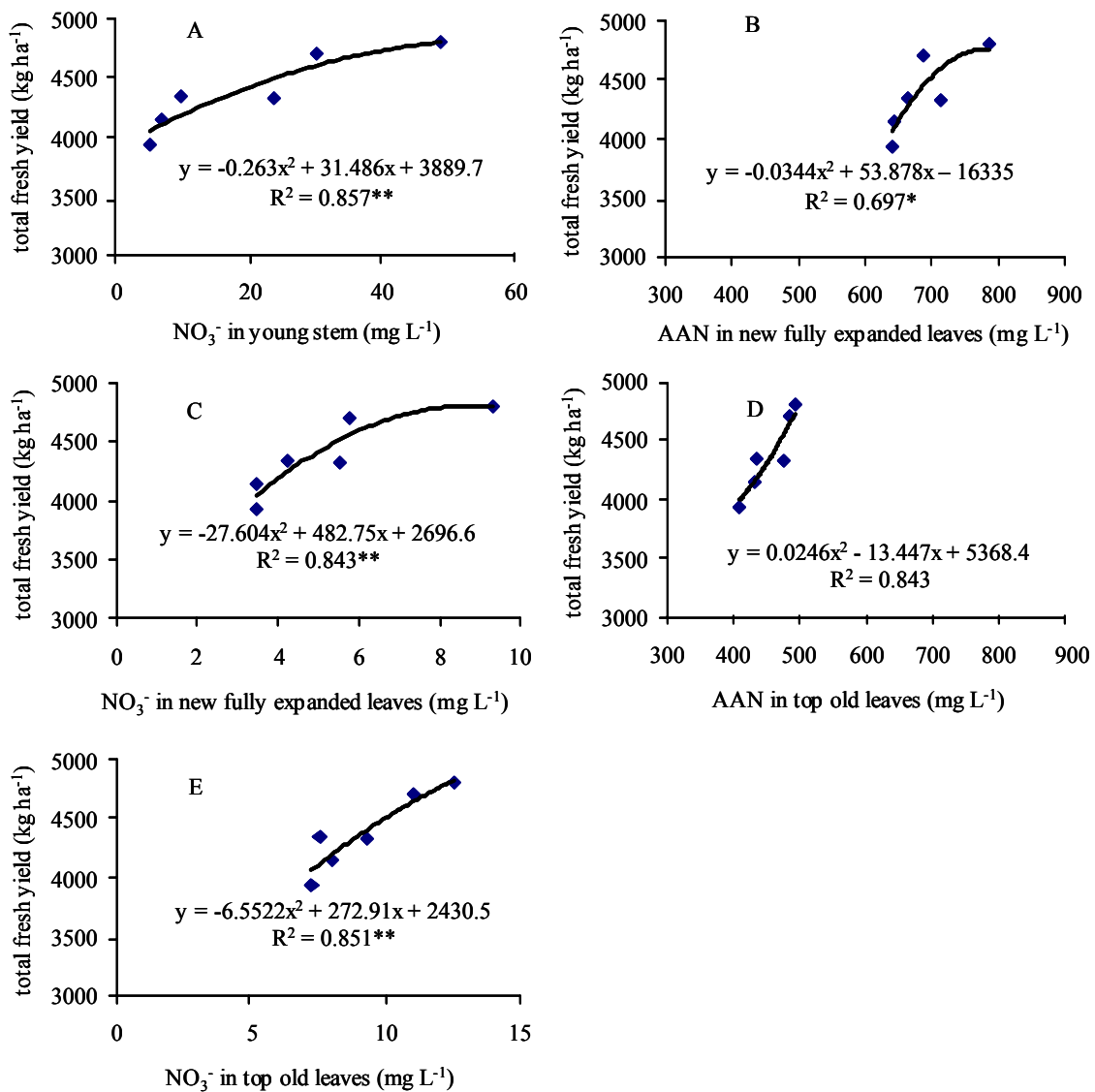


Fig. 3.11 The relationship between total fresh yield and different indicators in spring of 2003. (Top old leaves were the leaves that were growing on the brown stems and grew up from the former year).

spring. In autumn, total fresh yield had strongly significant correlations with  $\text{NO}_3^-$  and AAN in young stem and mature leaves (Fig. 3.12 A,  $R^2=0.904^{**}$ ; B,  $R^2=0.847^{**}$ ; C,  $R^2=0.922^{**}$ ; D,  $R^2=0.920^{**}$ ).

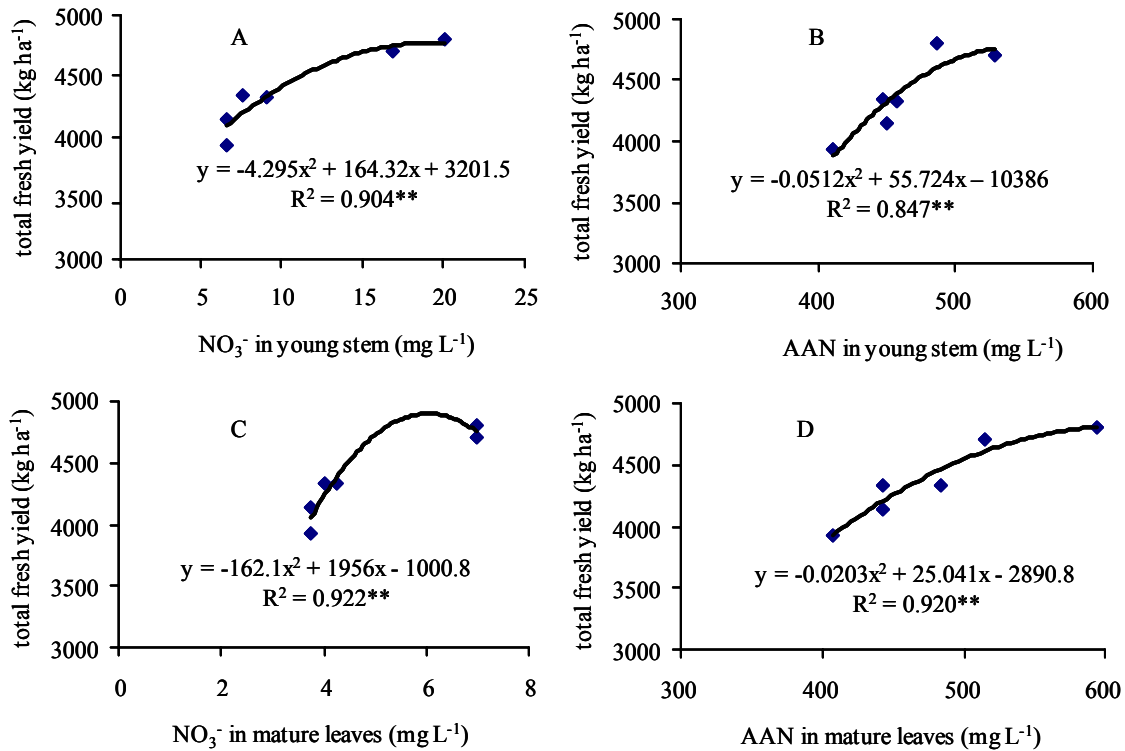


Fig. 3.12 The relationship between total fresh yield and different indicators in autumn of 2003.

The responses of the quality to different indicators were shown in Fig. 3.13 and Fig. 3.14. In spring, TP/AA ratio (the ratio of tea polyphenols to amino acids) was significantly correlated with  $\text{NO}_3^-$  in young stem (Fig. 3.13 A,  $R^2=0.662^*$ ),  $\text{NO}_3^-$  in new fully expanded leaves (Fig. 3.13 C,  $R^2=0.637^*$ ),  $\text{NO}_3^-$  in top old leaves (Fig. 3.13 E,  $R^2=0.615^*$ ) and AAN in new fully expanded leaves (Fig. 3.13 B,  $R^2=0.664^*$ ) and AAN in top old leaves (Fig. 3.13 D,  $R^2=0.930^{**}$ ). In autumn, TP/AA ratio showed significant correlations to  $\text{NO}_3^-$  in mature leaves (Fig 3.14 C,  $R^2=0.579^*$ ), AAN in young stem (Fig. 3.14 B,  $R^2=0.873^{**}$ ) and AAN in mature leaves (Fig 3.14 D,  $R^2=0.576^*$ ) except  $\text{NO}_3^-$  in young stem (Fig. 3.14 A,  $R^2=0.520$ ). These results indicated that the suitable indicators not only could index the yield levels, but also could index the quality of green tea.

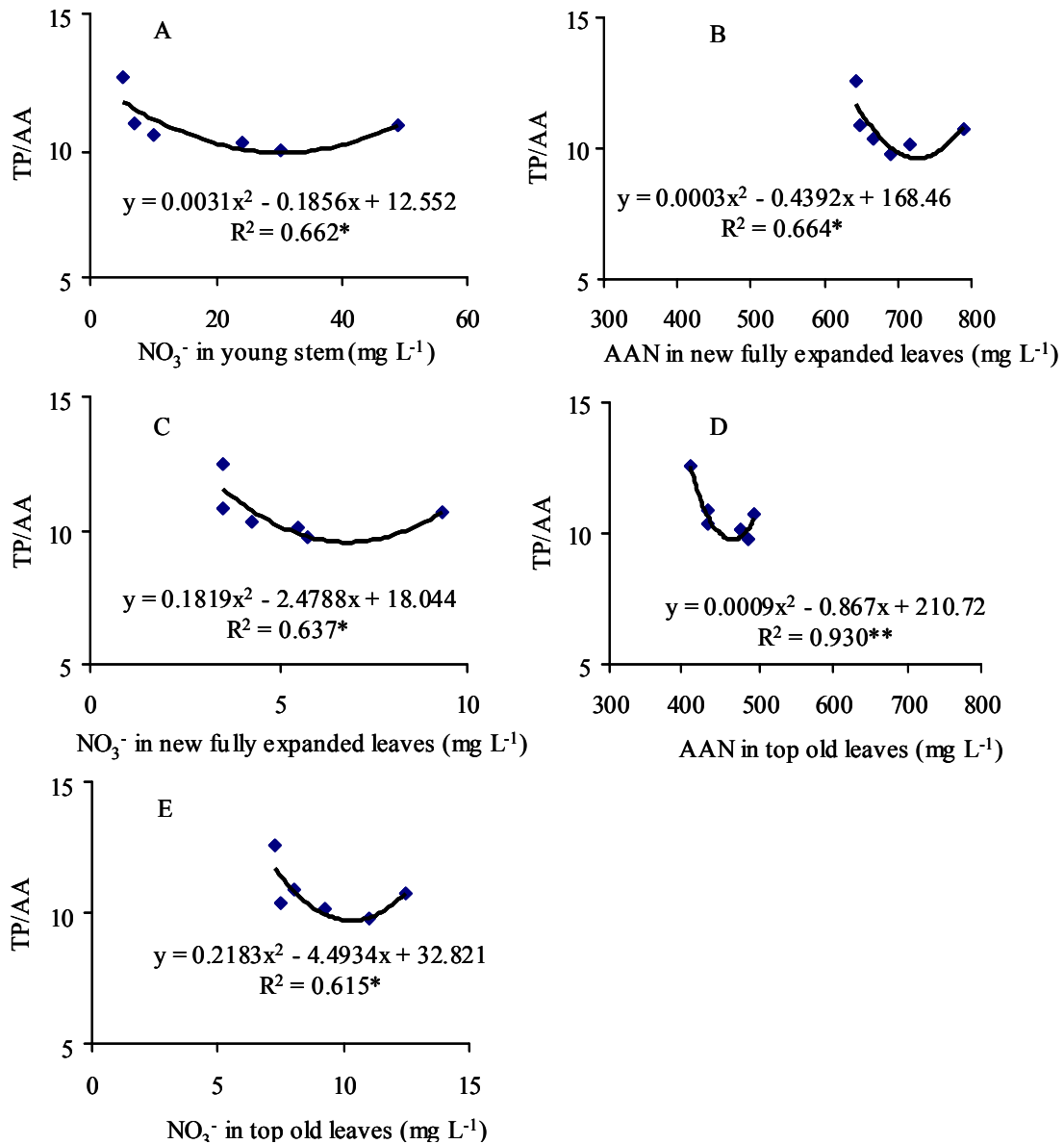


Fig. 3.13 The relationship between TP/AA ratio (tea polyphenols to amino acids) and different indicators in spring of 2003. (top old leaves were the leaves that were growing on the brown stems and grew up from the former year).

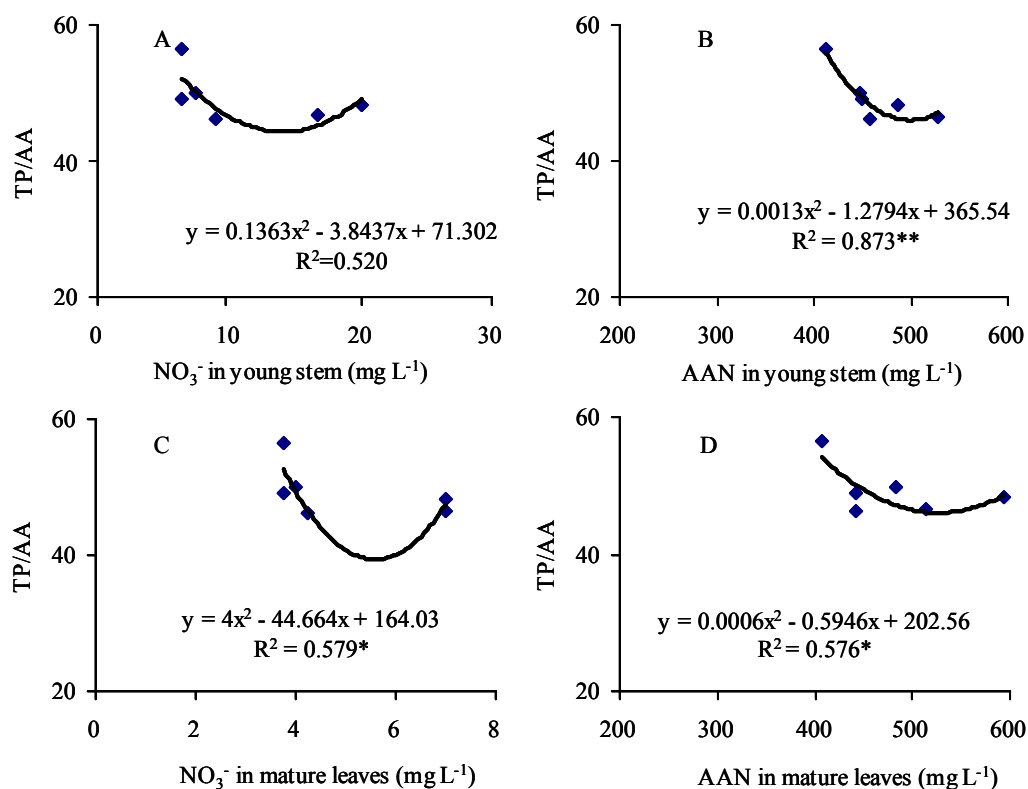


Fig. 3.14 The relationship between TP/AA ratio (tea polyphenols to amino acids) and different indicators in autumn of 2003.

In summer, using a quadratic polynomial function to describe the relationship between total fresh yield and indicators (Fig. 3.15 and Fig. 3.16), it becomes evident that only the  $\text{NO}_3^-$  in young stem was significantly correlated with total fresh yield under both shaded and exposed conditions (Fig. 3.15 A,  $R^2=0.974^{**}$ ; B,  $R^2=0.803^{**}$ ). Meanwhile, the relationships between TP/AA and indicators indicated that TP/AA under shaded and exposed conditions were only significantly correlated with  $\text{NO}_3^-$  in young stem (Fig. 3.16 A,  $R^2=0.824^{**}$ ; B,  $R^2=0.837^{**}$ ). Although fresh yield and quality under shading were all significantly correlated with AAN in young stem and  $\text{NO}_3^-$  and AAN in mature leaves (Fig. 3.15 C, E and Fig. 3.16 C, E, G), the relationships of the fresh yield and quality in full sun to them were not significant (Fig. 3.15 D, F, G, H and Fig. 3.16 D, F, H). According to the results of Fig. 3.15-3.16,  $\text{NO}_3^-$  in young stem was the only available indicator among plant indicators in summer.

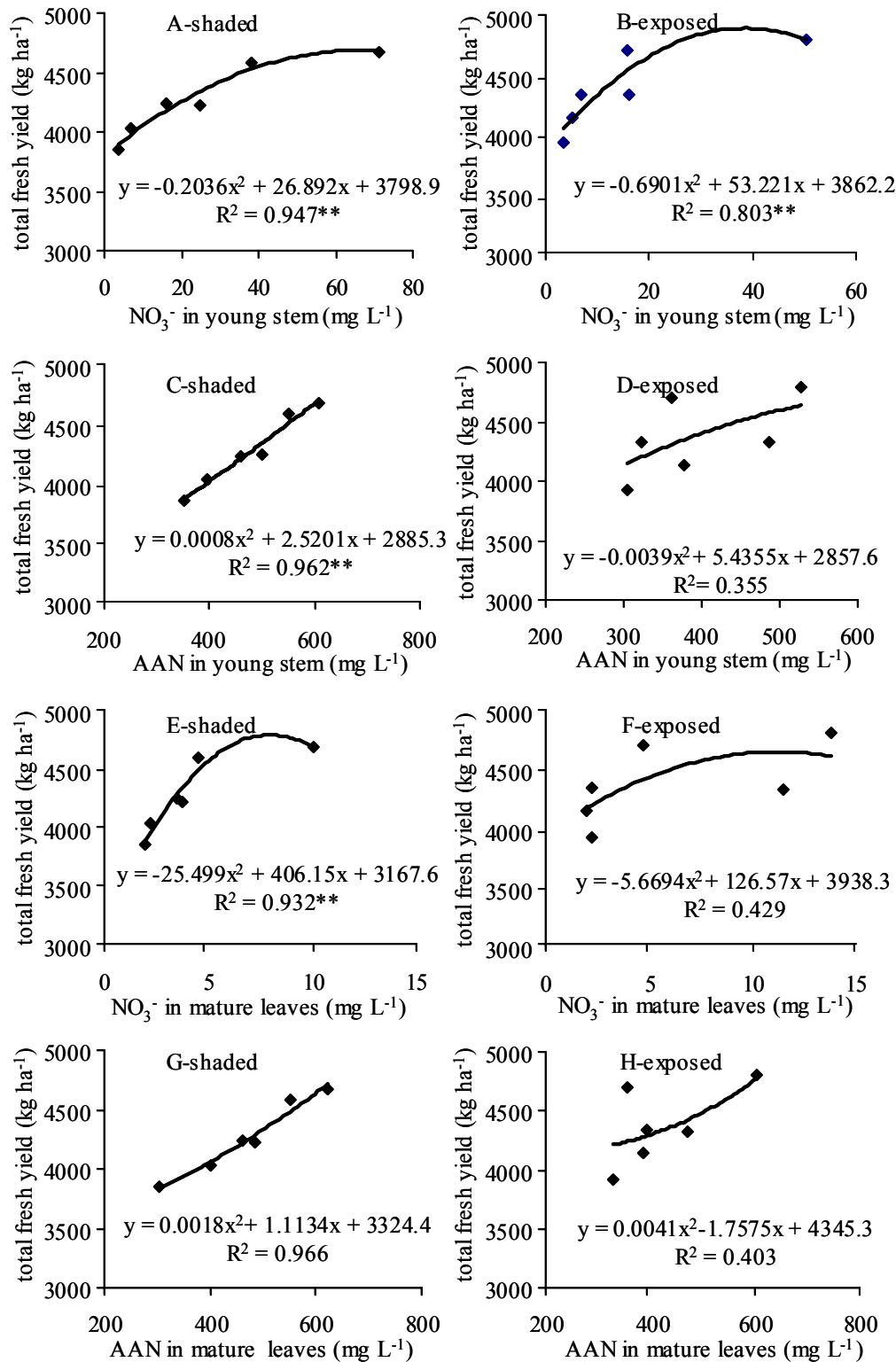


Fig. 3.15 The relationship between total fresh yield and nitrate (NO<sub>3</sub><sup>-</sup>) and amino acid nitrogen (AAN) in young stem and mature leaves under shaded and exposed conditions (A, B: NO<sub>3</sub><sup>-</sup> in young stem; C, D: AAN in young stem; E, F: NO<sub>3</sub><sup>-</sup> in mature leaves; G, H: AAN in mature leaves).

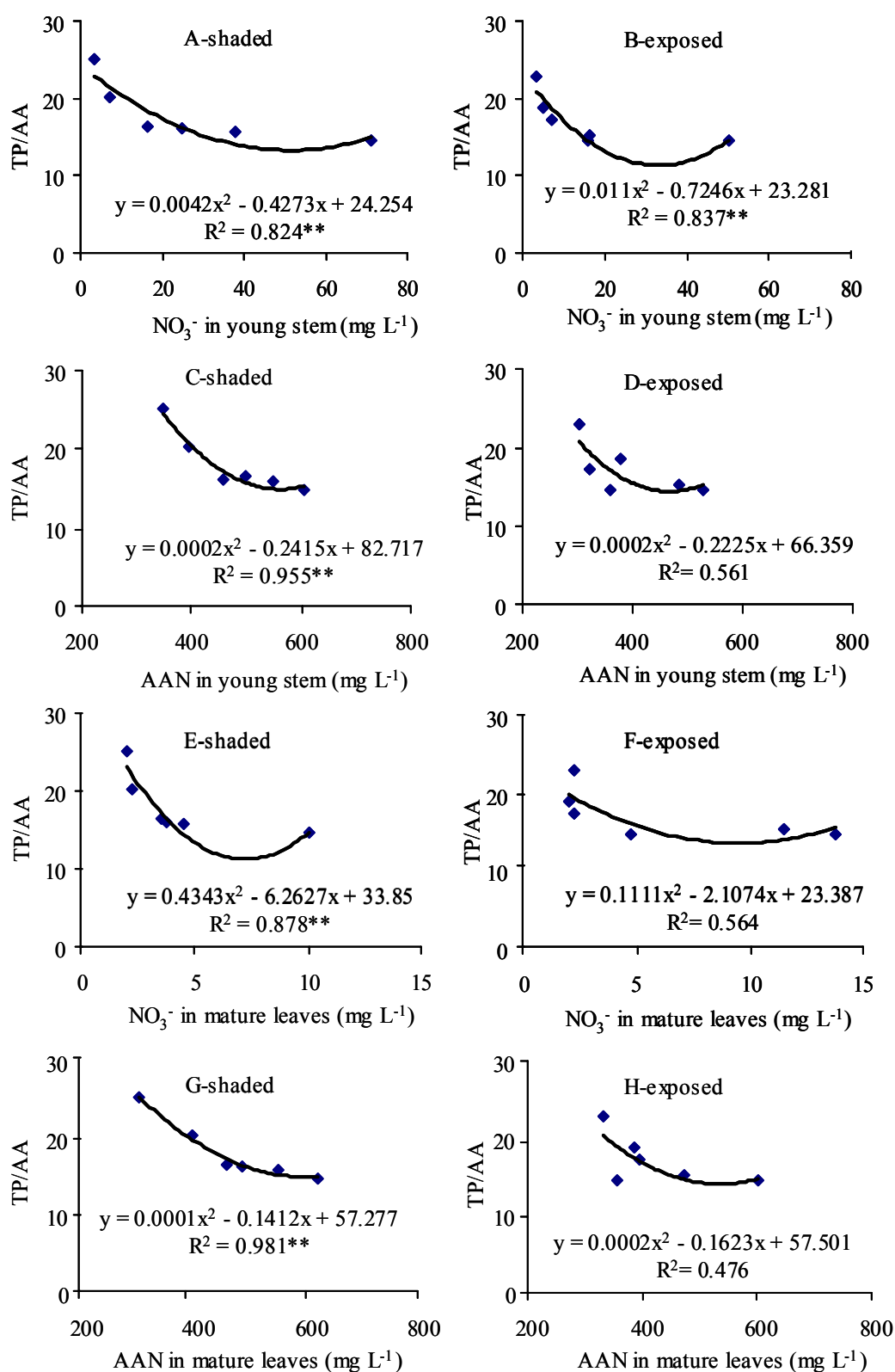


Fig. 3.16 The relationship between TP/AA and nitrate ( $\text{NO}_3^-$ ) and amino acid nitrogen (AAN) in young stem and mature leaves under shaded and exposed conditions (A, B:  $\text{NO}_3^-$  in young stem; C, D: AAN in young stem; E, F:  $\text{NO}_3^-$  in mature leaves; G, H: AAN in mater leaves).



The critical levels of the plant indicators were calculated from the functions in Fig. 3.11 A, B, C, E, Fig. 3.12 A-D and Fig. 3.15 A, B at 90% and 95% of the maximum yield as well as the functions in Fig. 3.13A-E, Fig. 3.14 A-D and Fig. 3.16 A, B at the lowest TP/AA (the best quality) and shown in Table 3.2. It was evident that the indicator contents at 95% of the maximum yield were closer to those at lowest TP/AA than those at 90% of the maximum yield. Shading had strong effect on indicator contents in summer, 32-51 mg L<sup>-1</sup> under shading and 20-33 mg L<sup>-1</sup> in full sun.

Table 3.2 The NO<sub>3</sub><sup>-</sup> and AAN (amino acid nitrogen) contents in different parts of the tea plant calculated from 90% of the maximum yield and 95% of the maximum yield as well as from the lowest TP/AA ratio in spring and autumn of 2003 (NEFL: new fully expanded leaves; TOL: top old leaves, which were growing on the brown stems and grew up from the former year; ML: mature leaves)

Indicators	NO <sub>3</sub> <sup>-</sup> or AAN content (mg·L <sup>-1</sup> )					
	90% Y <sub>max</sub>		95% Y <sub>max</sub>		the lowest TP/AA	
	in spring					
NO <sub>3</sub> <sup>-</sup> in young stem	17		33		30	
NO <sub>3</sub> <sup>-</sup> in NFEL	5		6		7	
NO <sub>3</sub> <sup>-</sup> in TOL	9		11		10	
AAN in NFEL	675		714		732	
AAN in TOL*	-		-		482	
	in autumn					
NO <sub>3</sub> <sup>-</sup> in young stem*	9		13		14	
NO <sub>3</sub> <sup>-</sup> in ML	4		5		6	
AAN in young stem*	454		485		492	
AAN in ML	464		510		495	
	in summer					
	shaded	exposed	shaded	exposed	shaded	exposed
NO <sub>3</sub> <sup>-</sup> in young stem	18	12	32	20	51	33

\*: The item with asterisk means that the indicators were not suitable to the diagnosis of tea plants.

### 3.3.4. Plant indicators and soil $N_{\min}$ in 0-60 cm soil depth

**In spring:** Soil  $N_{\min}$  was often used as the index to describe available N pool in the soil and soil  $N_{\min}$  in 0-60 cm soil depth could positively reflect the N application rate (Fig. 3.17  $R^2=0.989^{**}$ ).  $\text{NO}_3^-$  content in young stem (Fig. 3.18 A,  $R^2=0.964^{**}$ ), new fully expanded leaves (Fig. 3.18 B,  $R^2=0.968^{**}$ ) and top old leaves, which were growing on the brown stems and grew up from the former year (Fig. 3.18 D,  $R^2=0.897^{**}$ ) were all significantly positively correlated with soil  $N_{\min}$  in 0-60 cm soil depth. AAN contents in new fully expanded leaves (Fig. 3.18 C,  $R^2=0.903^{**}$ ) and in top old leaves (Fig. 3.18 E,  $R^2=0.783^{**}$ ) were also significantly correlated with soil  $N_{\min}$ . But the correlations of AAN in leaves with soil  $N_{\min}$  were not as significant as those of  $\text{NO}_3^-$  in leaves. All of these indicators could reflect the available nitrogen level in soil (Fig. 3.18).

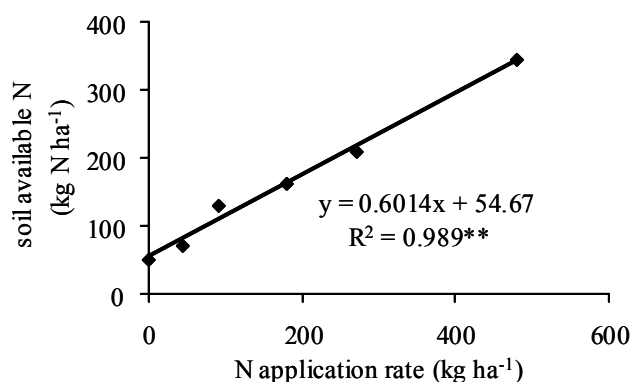


Fig. 3.17 The relationship between nitrogen application rate and soil available N in 0-60 cm soil depth in spring of 2003. 30% of N for each treatment was applied on February 25 and the soil samples were taken on April 22.

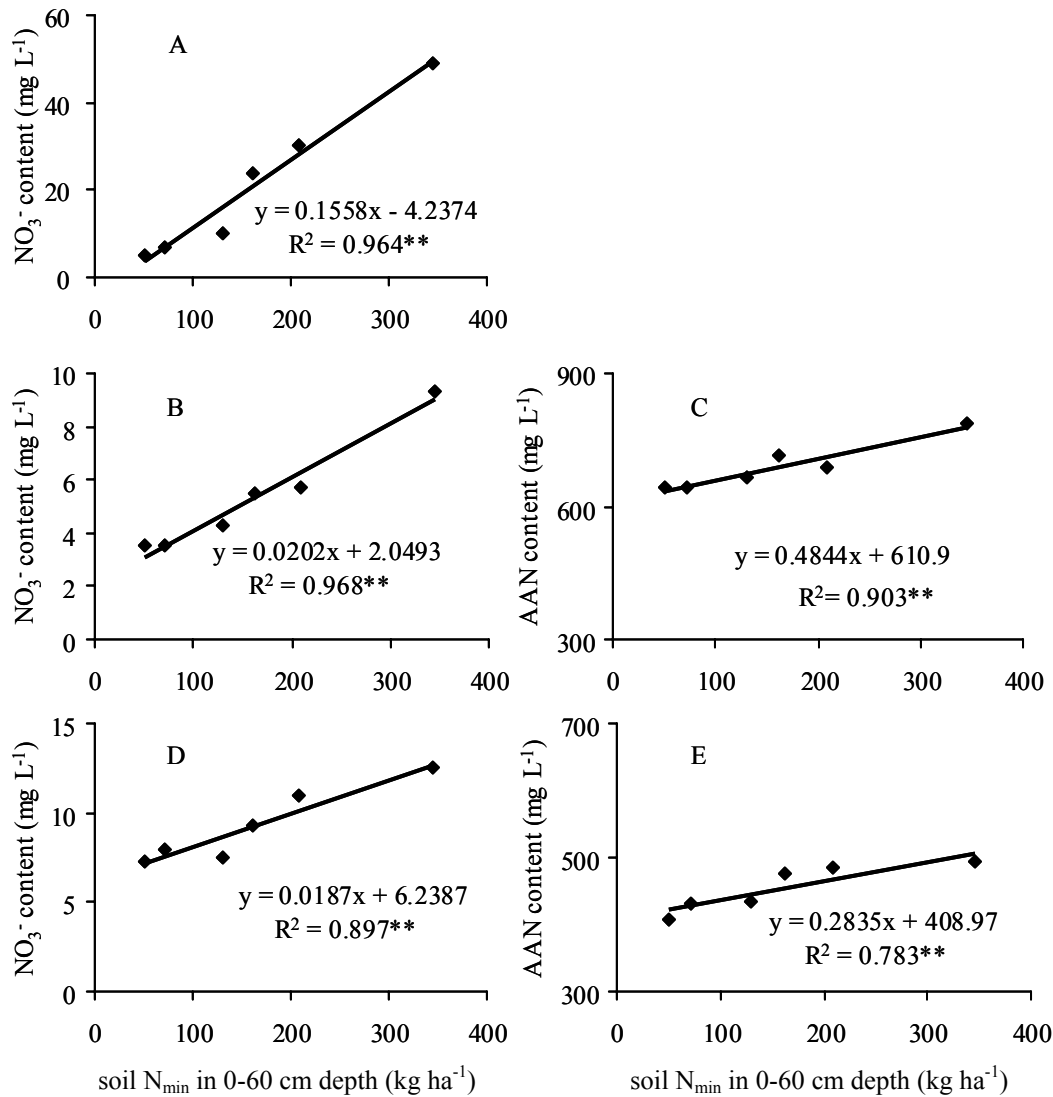


Fig. 3.18 The relationship between nitrate and/or amino acid nitrogen in young stem, new fully expanded leaves, top old leaves and soil  $N_{\min}$  in spring of 2003. (A: nitrate content in the young stem; B, C: nitrate content and amino acid nitrogen (AAN) in new fully expanded leaves; D, E: nitrate content and amino acid nitrogen (AAN) in top old leaves, which were on the brown stems and grew up from the former year.

**In autumn:** Soil  $N_{\min}$  in 0-60 cm soil depth was significantly correlated with N application rates (Fig. 3.19,  $R^2=0.895^{**}$ ), though the correlation of soil  $N_{\min}$  with N application rates was not as significant as that in spring.  $\text{NO}_3^-$  in young stem (Fig. 3.20 A,  $R^2=0.666^*$ ) was significantly correlated with soil  $N_{\min}$ . But AAN in young stem had no significant correlation with soil  $N_{\min}$  (Fig. 3.20 B,  $R^2=0.317$ ), which indicated that AAN in young stem was not a good indicator. Combination with Fig. 3.14 A, young stem was

not a good organ as N diagnosis in autumn.  $\text{NO}_3^-$  and AAN in mature leaves were both highly correlated with soil  $\text{N}_{\text{min}}$  (Fig. 3.20 C,  $R^2=0.510^*$ ; D,  $R^2=0.777^{**}$ ). Generally, the correlations between  $\text{NO}_3^-$  and AAN in different organs and soil  $\text{N}_{\text{min}}$  in autumn were not as good as those in spring.

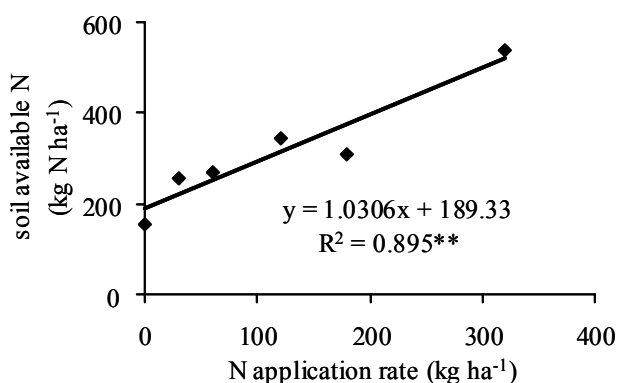


Fig. 3.19 The relationship between nitrogen application rate and soil available N in 0-60 cm soil in the autumn of 2003. 20% of N for each treatment was applied on August 22 and the soil samples were taken on September 20.

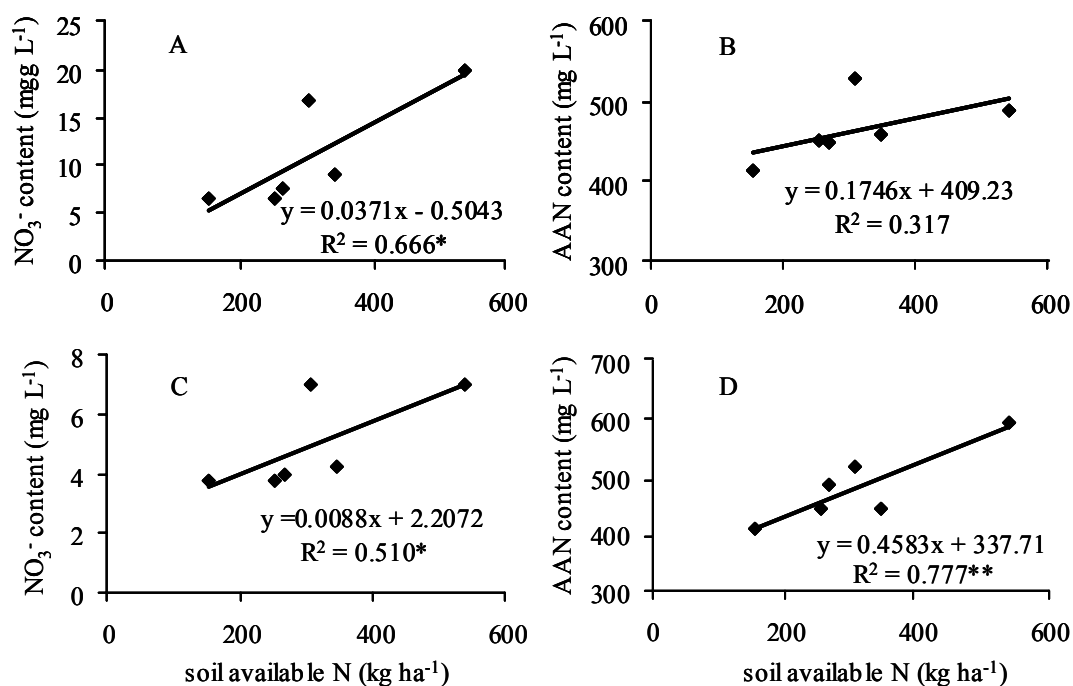


Fig. 3.19 The relationship between nitrate and amino acid nitrogen in young stem and mature leaves and soil available N in 0-60 cm soil in autumn of 2003. (A, B: nitrate content and amino acid nitrogen (AAN) in young stem; C, D: nitrate content and amino acid nitrogen (AAN) in mature leaves).

**In summer:** The relationship between soil  $N_{\min}$  in 0-60 cm soil depth and N application rates was shown in Fig 3.21. They were highly linearly correlated ( $R^2=0.970^{**}$ ). The N application rates were reflected proportionally by soil  $N_{\min}$ .

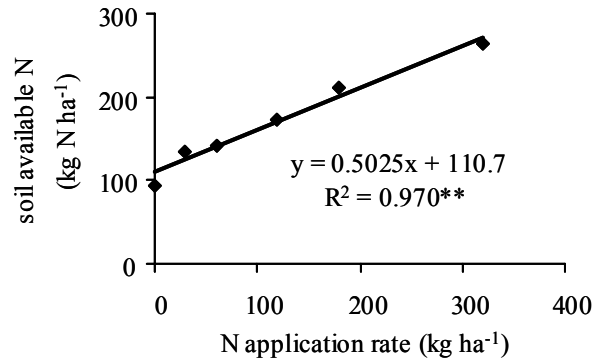


Fig. 3.21 The relationship between soil available N in 0-60 cm depth soil and N application rate in summer of 2003. 20% of N for each treatment was applied after pruning on May 23 and the soil samples were taken on August 13 after shading for 15 days.

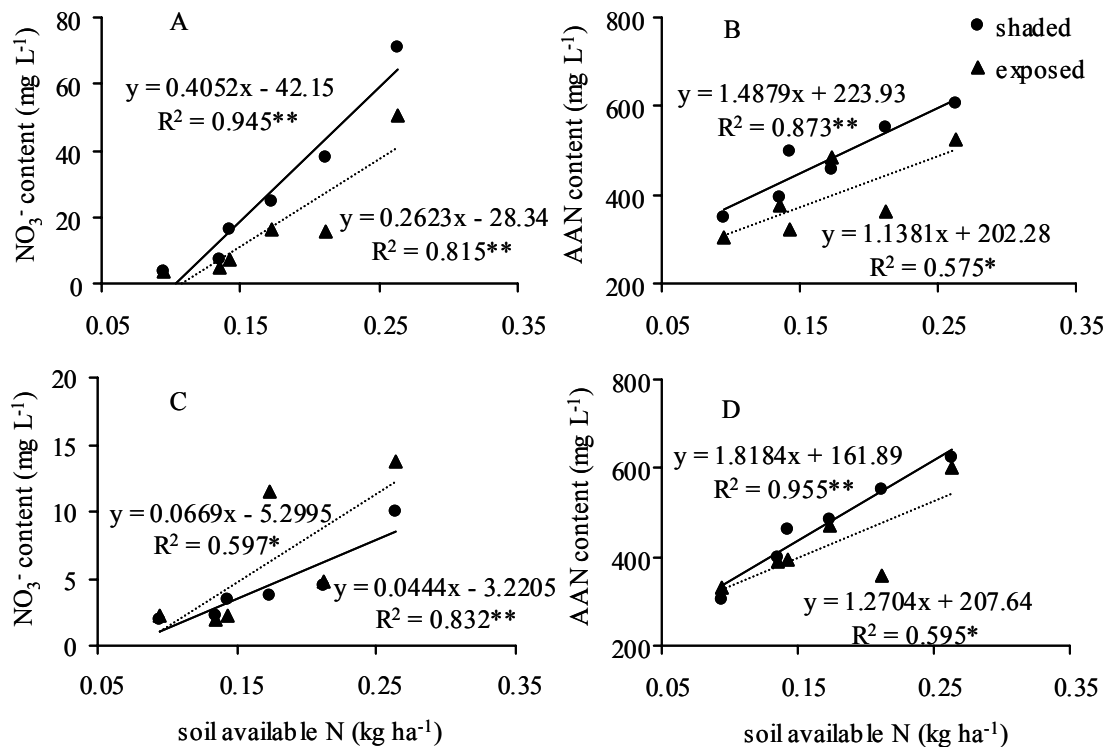


Fig. 3.22 The relationships between the contents of nitrate ( $NO_3^-$ ) and amino acid nitrogen (AAN) in young stem and in mature leaves and soil available N in 0-60 cm soil depth under shaded and exposed conditions (A, B:  $NO_3^-$  and AAN in young stem; C, D:  $NO_3^-$  and AAN in mature leaves).

$\text{NO}_3^-$  and AAN contents in young stem and in mature leaves in both shaded and exposed conditions were all significantly linearly correlated with soil  $N_{\min}$  (Fig. 3.22). But the linear regression of  $\text{NO}_3^-$  in young stem to soil  $N_{\min}$  (Fig. 3.22 A, shaded:  $R^2=0.945^{**}$ ; exposed:  $R^2=0.815^{**}$ ) was more significant than that of AAN in young stem (Fig. 3.22 B, shaded:  $R^2=0.873^{**}$ ; exposed:  $R^2=0.575^*$ ).  $\text{NO}_3^-$  and AAN in mature leaves under shading (Fig. 3.22 C, shaded:  $R^2=0.832^{**}$ ; D, shaded:  $R^2=0.955^{**}$ ) were more tightly correlated with soil  $N_{\min}$  than those in full sun (Fig. 3.22 C, exposed:  $R^2=0.597^*$ ; D, exposed:  $R^2=0.595^*$ ).

### 3.3.5. Critical levels of plant indicators and soil $N_{\min}$ as well as the optimum N application rate in different seasons

All of the available critical contents of plant indicators ( $\text{NO}_3^-$  and AAN in different organs) in three seasons in Table 3.2 were fitted back to the linear curves shown in Fig. 3.18, Fig. 3.20 and Fig. 3.22, respectively. The corresponding critical soil  $N_{\min}$  values in 0-60 cm soil depth at critical or sufficient indicator contents were calculated and shown in Table 3.3. Not the critical soil  $N_{\min}$  values at 90% of the maximum yield but those at 95% of maximum yield were much closer to those at the lowest TP/AA ratio. According to the results in Table 3.3, the critical soil  $N_{\min}$  was 200-260 kg N ha<sup>-1</sup> in spring, 320-430 kg N ha<sup>-1</sup> in autumn. In summer, shading had no effect on critical soil  $N_{\min}$ , 180-230 kg N ha<sup>-1</sup> in both shaded and exposed conditions.

Fit the critical soil  $N_{\min}$  values of 200-260 kg N ha<sup>-1</sup> in spring, 320-430 kg N ha<sup>-1</sup> in autumn and 180-230 kg N ha<sup>-1</sup> in summer in Table 3.3 back into the linear curve in Fig. 3.17, Fig. 3.19 and Fig. 3.21, respectively. The optimum N application rate within the season ( $N_{\text{opts}}$ ) according to soil  $N_{\min}$  was 240-340 kg N ha<sup>-1</sup> in spring, 130-230 kg N ha<sup>-1</sup> in autumn and 140-240 kg N ha<sup>-1</sup> in full sun and under shading in summer, respectively.

Table 3.3 The critical soil  $N_{\min}$  in 0-60 cm soil depth calculated from the contents of plant indicators at 90% of the maximum yield ( $90\%Y_{\max}$ ), at 95% of maximum yield ( $95\%Y_{\max}$ ) and at the lowest TP/AA ratio (the ratio of tea polyphenols to amino acids) (NEFL: new fully expanded leaves; TOL: top old leaves, which were growing on the brown stems and grew up from the former year; ML: mature leaves).

	critical soil $N_{\min}$ in 0-60 cm depth ( $\text{kg N ha}^{-1}$ )					
	$90\%Y_{\max}$		$95\%Y_{\max}$		The lowest TP/AA	
	in spring					
$\text{NO}_3^-$ in young stem	136		239		220	
$\text{NO}_3^-$ in NFEL	146		196		245	
$\text{NO}_3^-$ in TOL	148		255		201	
AAN in NFEL	132		213		250	
	in autumn					
$\text{NO}_3^-$ in ML	204		317		431	
AAN in ML	276		376		343	
	in summer					
	shaded	exposed	shaded	exposed	shaded	exposed
$\text{NO}_3^-$ in young stem	148	154	183	184	230	234

## 4. Discussion

### 4.1. Yield and the optimum N rate

Yield, quality and economic profit are the three most important aspects to green tea. In this study, N application rate had no significant effect on the yield, but the yield in 2003 was more sensitive to N application rate (Fig. 3.1 and Fig. 3.2). N fertilization rate in tea is very high and may exceed  $3000 \text{ kg N ha}^{-1} \text{ a}^{-1}$  (Watanabe, 1995). Considering the relatively low amount of N removal by plucking from the production system ( $40\text{-}80 \text{ kg N ha}^{-1} \text{ a}^{-1}$ , Fig. 3.3), the ecological hazard caused the attention. In many different crops, it has been tested not to go to the maximum yield, but for 90% or 95% of the value (Baethgen and Alley, 1989; Li et al., 1997; Fageria and Baligar, 2001). By this, not only the amount of N fertilizer applied can be reduced substantially, but also the  $\text{NO}_3^-$  content too. However, in tea, this is not economically since the quality is markedly correlated with the contents of

total nitrogen and amino acids (Watanabe, 1995) and the profit of tea growers is strongly related to the yield and quality. According to the investigation of the tea price in 500 sites in Zhejiang province from 1996 to 2000, the domestic price of made tea ranged from 9 to 167 RMB kg<sup>-1</sup> made tea (55 RMB kg<sup>-1</sup> in an average, only seven of the investigated tea prices within 5 years were below 22 RMB kg<sup>-1</sup>) (Agricultural Provincial Government Department of Zhejiang province, personal communication). For example, if the tea price of 22 RMB kg<sup>-1</sup> made tea is used to calculate, the profit after the additional N application (372 kg N ha<sup>-1</sup> a<sup>-1</sup>) was 320 RMB ha<sup>-1</sup> a<sup>-1</sup> in Table 3.1. However, if the domestic tea price of 167 RMB kg<sup>-1</sup> made tea is taken, then the profit in Table 3.1 will be 9100 RMB ha<sup>-1</sup> a<sup>-1</sup>. These indicated that in order to obtain the high economic profit, it is necessary to consider the 95% of the maximum yield, especially when the price per unit made tea is high. So the optimum rate of N fertilizer within a year ( $N_{\text{opty}}$ ) in this study was the rate that resulted in 95% of the maximum yield with an adaptation of the definition of Fageria and Baligar (2001). 810 kg N ha<sup>-1</sup> a<sup>-1</sup> of  $N_{\text{opty}}$  was obtained according to this yield level. In summer, shading reduced  $N_{\text{opty}}$  (700 kg N ha<sup>-1</sup> a<sup>-1</sup>) at the same time of decreasing the yield (Fig. 3.2). These  $N_{\text{opty}}$  are in the range of 600-900 kg N ha<sup>-1</sup> a<sup>-1</sup> for the production of the famous and high quality green tea in China (experienced value in TRI, personal communication)

#### 4.2. Comparison of indicators

The selection of indicators was an essential first step to establish the system of diagnosis. NO<sub>3</sub><sup>-</sup>-N content was initially used as an index to study the N nutrition of spring wheat by Gardner and Jackson (1976), though no critical NO<sub>3</sub><sup>-</sup>-N level was established at that time, they thought if the NO<sub>3</sub><sup>-</sup>-N content was lower than 2000 mg kg<sup>-1</sup>, this indicated that N was insufficient to spring wheat. Before maturity, if the NO<sub>3</sub><sup>-</sup>-N content in wheat at any growth stage was lower than this value, deficiency symptoms would emerge and had some effect on the yield. When the nutrition was diagnosed, if the critical level was calculated from the relationship between nutrition content in plant tissue and the final yield, this kind of work was called “prognosis”; if the critical level was calculated from the relationship between nutrition content in plant tissue and the biomass at that time, it was called instantaneous diagnosis, that is, exactly, “diagnosis” (Li et al., 1997). In most production,



prognosis was applied. Even if the name differences existed, in this study, diagnosis was still used to describe this part of work.

#### 4.2.1. Responses of plant indicators to soil $N_{\min}$

The regression of total yield and quality with soil  $N_{\min}$  indicated that soil  $N_{\min}$  in spring, autumn and summer was a good indicator (Fig. 3.7 and Fig. 3.8). But the smaller differences in soil  $N_{\min}$  under shading and in full sun evidently indicated that it was not a sensitive indicator. Contrarily, plant indicator,  $\text{NO}_3^-$  in young stems showed big differences under shading and in full sun (Table 3.2). The comparison of plant analysis suggested that in spring,  $\text{NO}_3^-$  measured with reflectrometer (nitrate test strips) in young stem and in new fully expanded leaves as well as in the top old leaves could reflect the N in soil available to plant (Fig. 3.18 A, B, D). Because in the very early spring, no young stem and no new fully expanded leaves could be used, the top old leaves were taken to check if they could be used as the diagnosis part at that time. The results of AAN in new fully expanded leaves and top old leaves also demonstrated that AAN was also a good indicator to N diagnosis (Fig. 2.18 C, E). However, in autumn, AAN in young stem was not a good indicator any more (Fig. 3.20 B,  $R^2=0.317$ ). Compared to the  $\text{NO}_3^-$  in mature leaves, AAN in mature leaves in autumn was more suitable as the indicator with highly linear correlation (Fig. 3.20 D,  $R^2=0.777^{**}$ ). In summer,  $\text{NO}_3^-$  in young stem in full sun and under shading was significantly linearly correlated with soil  $N_{\min}$  (Fig. 3.22). Generally, plant indicators were significantly linearly correlated with soil  $N_{\min}$ . Therefore, plant analysis could not only index the yield and quality (Fig. 3.11-3.16), but also could index soil  $N_{\min}$ . Compared to soil analysis, plant analysis is a faster method and it was also regarded a more sensitive indicator (Lacroix and Cousin, 1997).

#### 4.2.2. Responses of yield and quality to plant indicators

To establish the relationship between total fresh yield and the different indicators is the next key step of diagnosis. AAN in top old leaves had a converse quadratic correlation compared to the others, though there was a high correlation with total fresh yield in spring (Fig. 3.11 D). However,  $\text{NO}_3^-$  in the top old leaves was highly correlated with the total

fresh yield (Fig. 3.11 E), which indicated that in the very early spring, not AAN but  $\text{NO}_3^-$  in the top old leaves could be used as the indicator. Meanwhile, this result also implied that the diagnosis in the very early spring was not so necessary just after a large amount of N fertilizer was applied to improve the growth of the buds because of the speciality of tea plant and the importance of green tea flushes in spring. However, TP/AA ratio, which showed significantly negative linear regression with green tea quality (Shi and Liu, 1987), exhibited a highly significant correlation with AAN in top old leaves (Fig 3.13 D). This further indicated that tea flushes were the most important sink in the very early spring, the top old leaves worked as the main functional leaves, therefore it showed a very close correlation with quality. But it could not be used as the diagnosis part to predict the yield, especially for the AAN in it (Fig. 3.11 D). In autumn, different indicators showed highly significant correlations with yield (Fig. 3.12). But if combined the relationships between TP/AA ratio and indicators (Fig 3.14 A),  $\text{NO}_3^-$  in young stem would not be the suitable indicator in autumn. Considering the relationship between AAN in young stem and soil  $\text{N}_{\text{min}}$  in autumn (Fig. 3.20 B), the conclusion that young stem in autumn was not a good diagnosis part could be made. Because in the early autumn, the young stem was not as sappy as that in late spring, most stems in tea plants were highly fibrous. So from the results shown in Fig. 3.20 B and Fig. 3.14 A, the possibility of the young stem as the diagnosis part in autumn was excluded. In summer, the relationships of total fresh yield and TP/AA ratio with indicators (Fig. 3.15 and Fig. 3.16) further verified that  $\text{NO}_3^-$  in young stem was a good and accurate indicator under shading condition.

### 4.3. Critical contents of plant indicators

The determination of critical level was the key step of diagnosis. The critical value was the nutrient content when the biomass (or yield) decreased significantly or deficiency symptom appeared. Comparison of the critical levels of the indicators at 90% and 95% of the maximum yield and at the lowest TP/AA (the best quality) (Table 3.2) indicated that from 90% $Y_{\text{max}}$  to 95% $Y_{\text{max}}$ ,  $\text{NO}_3^-$  in young stem was increased by 94% in spring and 44% in autumn by 5% $Y_{\text{max}}$  (the difference between 95% $Y_{\text{max}}$  and 90% $Y_{\text{max}}$ ), respectively. The  $\text{NO}_3^-$  content in new fully expanded leaves was increased by 20% in spring and the  $\text{NO}_3^-$

content in mature leaves were increased by 25% in autumn by  $5\%Y_{\max}$ , respectively. The AAN content in both seasons was increased much less than  $\text{NO}_3^-$  by  $5\%Y_{\max}$ . Generally, the content of indicators in spring were increased much more than those in autumn. The calculation of the indicators at the lowest TP/AA indicated that  $\text{NO}_3^-$  or AAN content at the lowest TP/AA were closer to the values calculated from  $95\%Y_{\max}$ . When the best quality was obtained, only the  $\text{NO}_3^-$  in young stem and in top old leaves in spring and AAN content in mature leaves in autumn were in the range of that from  $90\%Y_{\max}$  to  $95\%Y_{\max}$ . The others were all higher than that at  $95\%Y_{\max}$ , or close to those at  $95\%Y_{\max}$ , especially for  $\text{NO}_3^-$  in leaves in both seasons. All of these further indicated that the consideration of 95% of the maximum yield was necessary to obtain better quality. According to the standpoint that the critical level should be higher than the most suitable content to grow in order to make plants to get sufficient nutrition to prevent the yield from decreasing (Li et al., 1997), the conclusion could be made that in spring, the critical indicator levels were 30-33  $\text{mg L}^{-1} \text{NO}_3^-$  in the young stem, 6-7  $\text{mg L}^{-1} \text{NO}_3^-$  in new fully expanded leaves, 10-11  $\text{mg L}^{-1} \text{NO}_3^-$  in the top old leaves and 714-732  $\text{mg L}^{-1}$  AAN in new fully expanded leaves, respectively; in autumn, they were 5-6  $\text{mg L}^{-1} \text{NO}_3^-$  and 495-510  $\text{mg L}^{-1}$  AAN in mature leaves, respectively (Table 3.2).

In summer, shading had a strong effect on critical indicator content, 32-51  $\text{mg L}^{-1}$  under shading and 20-33  $\text{mg L}^{-1}$  in full sun (Table 3.2). The accumulation of  $\text{NO}_3^-$  and AAN in young stem under shading (Fig. 3.22 A, B) indicated that shading restricted the further metabolism of nitrogen, such as the synthesis of protein. On the contrary, the lower  $\text{NO}_3^-$  and higher AAN in mature leaves under shading as compared to exposed conditions again indicated that transient shading reduced the activity of sink and the requirement of the sink to  $\text{NO}_3^-$  ( $\text{NO}_3^-$ -N content was much higher than  $\text{NH}_4^+$ -N content in soil, Fig. A9).  $\text{NO}_3^-$  as one of important N sources was transported to the shoot through the long-distance transport of the xylem and was accumulated in the young stem. The amino acids formed was less consumed in its further metabolism, thus resulted in the accumulation of AAN in mature leaves. The results of Kaiser and Förster (1989) indicated that nitrate reduction by leaves or leaf tissues was light-dependent and low  $\text{CO}_2$  prevented the nitrate reduction in leaves. In the full sun, high photosynthetic rate (Table 3.14) caused a rapid N metabolism

of the leaf, as the initial N source,  $\text{NO}_3^-$  is assimilated and transformed to other N containing compounds rapidly. So under shading,  $\text{NO}_3^-$  was mainly accumulated not in mature leaves (Fig. 3.22 C) but in young stem (Fig. 3.22 A). However, in full sun, the absorbed  $\text{NO}_3^-$  was mainly transported to leaves (Fig. 3.22 C), but was not accumulated in young stem (Fig. 3.22 A). The effect of shading on AAN in young stem and mature leaves were consistent, that is, shading could improve the content of amino acid nitrogen, which testified that shading could improve the quality by increasing the content of amino acids again (Table 2.6 and Table 2.7).

#### **4.4. Critical soil $N_{\min}$ and the optimum N application rate**

The critical soil  $N_{\min}$  obtained from the critical values of plant indicators were shown in Table 3.3. Considering the quality reason, the values were taken from the lowest TP/AA to 95% of the maximum yield. So according to the diagnosis results, the critical soil  $N_{\min}$  values were 200-260 kg N ha<sup>-1</sup> in spring, 320-430 kg N ha<sup>-1</sup> in autumn and 180-230 kg N ha<sup>-1</sup> in summer, respectively. In summer, shading had no effect on soil  $N_{\min}$  (Table 3.3). The optimum soil  $N_{\min}$  value deduced from the relationships with total yield and quality was 200-220 kg N ha<sup>-1</sup>, 350-360 kg N ha<sup>-1</sup> and 220-230 kg N ha<sup>-1</sup> in spring, autumn and summer, respectively (Fig. 3.7 and Fig. 3.8). They were in the range of the critical soil  $N_{\min}$  values that were directly calculated from plant indicators. This indicated that plant indicators had a wider range of diagnosis than soil  $N_{\min}$ . One of the reasons why the critical soil  $N_{\min}$  in autumn was higher than that in spring and summer was the last fertilization of 20% of total N for each treatment after summer tea and less intensive plucking than that in spring.

According to this study, if the highest total content of N of 6.5%, 4.8% and 4.6% in tea flushes in spring, summer and autumn and the highest total content of N of 3.5% in pruned tea were considered to calculate N removal, N removal was 160-200 kg N ha<sup>-1</sup> a<sup>-1</sup> from control to the highest N level treatment by plucking and pruning within one year. The losses of N from tea production system in this study were almost twice as much as that in black tea production system (90-120 kg N ha<sup>-1</sup> a<sup>-1</sup>) (Wanyoko, 1981 in Owuor et al., 1987). This difference was mainly caused by the lower N content (3.0-3.4%) in tea flushes in his

study. Another possible reason was that a large amount of N was taken away by the pruned tea flushes in this study. But the pruned tea flushes were still kept in the field as the organic matter returned to the production system after pruning. Therefore, the N removal by plucking was only 40-80 kg N ha<sup>-1</sup> a<sup>-1</sup> (Fig. 3.3), which was less than that in black tea in Kenya (Wanyoko, 1981 in Owuor et al., 1987). Othieno (in Owuor, 1987) estimated that the efficiency of nitrogen uptake by the tea plants was less than 50% under Kenya tea growing conditions. Although the results in this study were not sufficient to calculate the exact N use efficiency in tea production system, considering the relatively low soil N<sub>min</sub> and N removal in this study, we might also make the same conclusion with Othieno (in Owuor, 1987) that large amounts of the applied N fertilizer nutrients was lost from the production system. Maybe the strong leaching in the normal high rainfall, especially in spring and the early summer in the South of China was one of the reasons.

Based on the critical soil N levels, the N<sub>opts</sub> were 240-340 kg N ha<sup>-1</sup> in spring, 130-240 kg N ha<sup>-1</sup> in autumn and 140-240 kg N ha<sup>-1</sup> in summer, respectively. If based on the N<sub>opty</sub> of 810 kg N ha<sup>-1</sup> a<sup>-1</sup>, 30% of which applied for spring tea was 240 kg ha<sup>-1</sup> and 20% of which applied for autumn tea was 160 kg N ha<sup>-1</sup>. Compared to the calculated N<sub>opts</sub> from the curve in each season, 240 kg N ha<sup>-1</sup> (30% of the N<sub>opty</sub>) only reached the lower limiting threshold of 240-340 kg N ha<sup>-1</sup> in spring. However, 160 kg N ha<sup>-1</sup> (20% of the N<sub>opty</sub>) was in the range of the limiting threshold of 130-240 kg N ha<sup>-1</sup> in autumn. 20% of the optimum N application rate within a year (N<sub>opty</sub>, 810 kg N ha<sup>-1</sup> a<sup>-1</sup> in full sun and 700 kg N ha<sup>-1</sup> a<sup>-1</sup> under shading) for summer tea were 160 kg N ha<sup>-1</sup> in full sun and 140 kg N ha<sup>-1</sup> under shading. They were in the range of 140-240 kg N ha<sup>-1</sup>, though they are close to the lower limiting threshold in summer. According to the study of Konishi et al. (1978) that the nitrogen absorbed in autumn enabled to maintain the mass of tea plant and that the nitrogen absorbed in spring contributed to the new tea flushes, relatively. The results of Watanabe (1988) indicated that the nitrogen absorbed in autumn affected the growth of new shoots and the nitrogen absorbed in spring affected the increase of the total nitrogen and total amino acid contents in new shoots. In this study, the partition of the N application rate in different seasons can be adapted correspondingly, increasing N application rate in the early spring to obtain the quality as good as possible and decreasing

N application rate for autumn tea. Because in autumn, the tea flushes is plucked much less intensively and the quality is not so good as that in spring, which means the profit to tea growers is much less than that in spring. The N removal by autumn tea was only 1.5-3.0 kg N ha<sup>-1</sup>, the nitrogen use efficiency is quite low in autumn. Therefore, 20% of the total N applied after summer tea to meet the requirement of the growth of autumn tea is too much. Moreover, the 30% of total N applied as basal N fertilizer in the late autumn is enough to meet the growth of the flushes and the maintenance of the mass of tea plants as described by Konishi et al. (1978) and Watanabe (1988). Therefore, the investment for autumn tea can be reduced and applied in the production of spring tea, which can bring more economic benefit for tea growers. Moreover, if the summer tea is plucked to make green tea, the N application rate for summer tea could be increased correspondingly.

The present study has shown that NO<sub>3</sub><sup>-</sup> and AAN in leaves in both spring and autumn are good indicators for the diagnosis of N status in tea plant and that the N application rate at 95% of the maximum yield is taken as the optimum N rate within the year because of the consideration to the economic reasons. Moreover, the quality was considered as one of the main factors determining the optimum N application rate and the diagnosis indicators in tea plants, which was a progress and made a beneficial supplement for the nutrient diagnosis not only for tea plants but also for other cash or non-cash crops. However, because of the strong fluctuation of the tea price in different seasons or other constantly changing reasons and difficulties to get a reasonable price for green tea, the economical optimum N application rate was not considered so much when the N<sub>opty</sub> was calculated. The effort to compensate the economical loss was made by comparing the difference between 90% and 95% of the maximum yield (Table 3.1). Although the relationship between indicators and soil N<sub>min</sub> was built, only the N<sub>opty</sub> and N<sub>opts</sub> could be presented. In this study, only some basic information on suitable diagnosis procedure was obtained, and more work is needed over a wider rang of sites and years to establish a model including the establishment of the instant relationships between soil N<sub>min</sub> and indicators, the consideration of economic factors to fully evaluate the response of tea plants to N fertilizer, the development of a new methodology to identify the more exact critical indicators of N which can be used to predict the N status for growing fields. The

establishment of the relationship of different light intensity and yield as well as quality, the establishment of the relationship between different light intensity and the diagnosis standards of the indicators were needed in a wider range of sites and years. Considering the low N removal, more attention should be paid to the management to increase N use efficiency in tea production system in the future.





**Chapter4****Green tea (*Camellia sinensis* L.) response to potassium and magnesium fertilization****Abstract**

13-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Longjing 43) and 36-year-old green tea trees (*Camellia sinensis* L., variety of clone Biyun) in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences were used for this study. Field experiments were carried out on Red Soil (Ultisol, typic plinthodults) in 2002 and 2003. The treatments consisted of 5 fertilizer treatments (Control, N, NP, NPK, NPKMg) and formed a 5×4 completely randomized block design with four replications.

The application of potassium and magnesium fertilizers could increase the yield and improve the quality, especially for Longjing43 in 2003. Due to the antagonism of K and Mg, the effect of K on increasing the content of amino acids and the ratio of ascorbic acid to dehydroascorbic acid was larger than K and Mg together. The effect of K and Mg on increasing yield and improving quality was inhibited by increased soil exchangeable Ca because of the antagonism of Ca, K and Mg.

**1. Introduction**

Potassium and magnesium are both essential elements of tea plant, they function in many physiological procedures. Potassium is one of the first elements to be reported to intervene with the water economy (Maurya and Gupta, 1984), pigment synthesis (Bolle-Jones and Notton, 1953), protein synthesis and enzyme activities, which are all important physiological functions in a plant system. Magnesium is not present in a large quantity in tea plant but it is linked with the formation of chlorophyll as the central atom of the chlorophyll molecule, photosynthesis and nitrogen metabolism (Marschner, 1995). So

potassium and magnesium can deeply affect the yield and improve the quality of green tea.

Tea plants are cultivated in many parts of humid and sub-humid tropical regions of the world mainly in acid soils having pH (H<sub>2</sub>O) of 4.5-5.5 (Othieno, 1992; Ranganathan and Natesan, 1985). Most of the tea soils with low pH are highly weathered. However, the gradual weathering and acidification of soils in these regions can accelerate the losses of potassium and magnesium from soil by leaching. Further, their insufficient supply results in elemental deficiencies in plants. In tea, of the mineral elements available in the mature leaf tissues, potassium comes next to nitrogen and amounts to 1.5 to 2.0 % on a dry matter basis (Krishnapillai and Ediriweera, 1986), and a typical analysis of the components of the two leaves and a bud shows that the mean magnesium content in hybrid seedling tea is 0.19% (Willson, 1975a). It might be expected that the supply of magnesium to tea would not be a matter of great concern, but the availability of all bases in typical acid tea soils is low and magnesium deficiency has caused some attention in the past. The earliest mention of magnesium deficiency was published in Nyasaland in 1932, the next being from Indonesia in 1941 (Willson, 1975a). It was reported that magnesium uptake was increased by liming acid soils, tea yields and the magnesium content of leaves being increased by the application of magnesium fertilizer together with a small amount of calcium. Analytical data for green leaves and leaves showing the symptoms of magnesium deficiency have been reported (Chenery and Schoenmakers, 1959). It has been shown that increased amounts of potassium or calcium in the soil reduced the quantity of magnesium in the leaves (Kawai and Ikegawa, 1960). But from the references available it is not clear whether this effect was noted on all the ages of leaf samples. Yield increases from the application of magnesium fertilizer to soil in which magnesium availability was similar to that of many East African soils have also been reported by Datuadze in 1964.

The antagonism between potassium and magnesium is well-known for many crops and this effect on the nutrient contents of coffee leaves has been demonstrated (Mulder, 1959 in Willson, 1975b). The possibility of heavy applications of potash fertilizers to tea restricting magnesium uptake has been discussed (Tolhurst, 1955) without quoting any examples. It was reported that the uptake of magnesium was reduced by increasing the

amount of potassium available in the soil but it is not clear whether this had any effect on yield. A reduction in yield following excessive application of magnesium fertilizer has been mentioned, which could result from a restricted uptake of potassium (Carpenter et al., 1919 in Willson, 1975a).

Nutrient deficiency in soils and poor fertilization are possibly two reasons for low yield and quality of tea (Malenga, 1994). With the fast development of tea production in China, great attention has been paid to the balanced fertilization in tea cultivation for good quality and high yield of tea in recent years. It is necessary to study the background of nutrients, the soil properties in tea-grown soils and their effects on tea quality and yield in order to increase fertilizer efficiency.

In China, preliminary studies indicated that Chinese tea soils were insufficiently supplied with potassium and magnesium except nitrogen and phosphorus and that these two nutrients became the main factors restricting the increase of tea yield and quality (Wu and Ruan, 1994). Field and pot experiments showed that the yield of different types of tea (black, green and oolong tea) responded significantly to potassium and magnesium applications, suggesting that the application of potassium and magnesium is urgently needed in order to improve both yield and quality (Wu and Ruan, 1994). However, the understanding of the effects of potassium and magnesium on tea quality and in particular on the content of free amino acids in tea leaves is very poor. Results from other crops indicated that amino acids accumulated in plants insufficiently supplied with potassium and magnesium due to the inhibition of protein synthesis (Mengel and Helal, 1968; Marschner, 1995). Therefore, the objective of the present study was to investigate the effects of N, P, K, Mg nutrients on the yield and quality of green tea and try to elucidate the relationship between the contents of nutrients, tea yield and quality, especially that of K and Mg.

## 2. Materials and Methods

13-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Longjing 43) and 36-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone

Biyun) in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, China (30°16' north latitude and 120°12' east longitude) were used for this study. Field experiments were carried out in 2002 and 2003. Soil type was Red Soil (Ultisol, typic plinthodults). The chemical properties of the soil are shown in Table 4.1 (for Longjing43) and Table 4.2 (for Biyun). The treatments consisted of 5 fertilizer treatments (Control, N, NP, NPK, NPKMg) and formed a 5×4 completely randomized block design with four replications. Urea was the source of fertilizer N and was applied as described in Chapter 1. 200 kg ha<sup>-1</sup> a<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 250 kg ha<sup>-1</sup> a<sup>-1</sup> K<sub>2</sub>O, and 50 kg ha<sup>-1</sup> a<sup>-1</sup> MgO were applied in the corresponding treatments as basal fertilizers. The resources were diammonium phosphate, potassium sulphate and magnesium sulphate, respectively. Sulphur powder was used as the sulphur source for the other treatments to reach the same sulphur level with that in NPKMg treatment.

The measurements of soil chemical properties, yield determination and quality of tea flushes are identical to Chapter 1. The measurement of mineral nutrient content and statistical analysis of data are identical to Chapter 2. The composition of amino acids was determined with amino acid analyser (Biochrom 30) according to Reins et al. (1991). The measurement of ascorbic acid took place as described in Chapter 2. Chlorophyll content (SPAD value) was measured with chlorophyll meter SPAD-502. However, the readings of SPAD meter for the leaves in autumn were out of the range of 0-99.9, which means the leaves in autumn are not suitable to be measured with SPAD meter, because they are dark green and too thick. Only the SPAD values in spring and summer were measured.

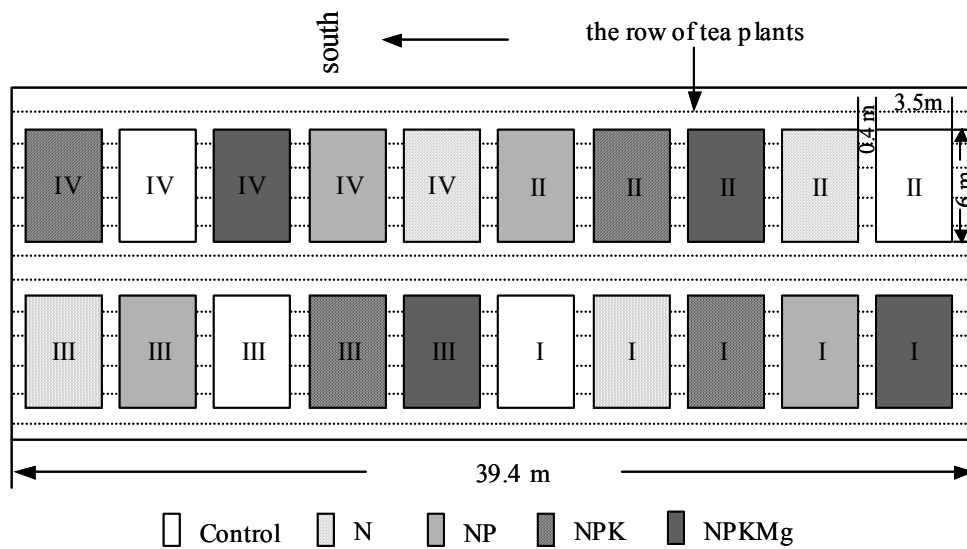


Fig. 4.1 Sketch of experimental field set-up for Longjing43 in tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China.

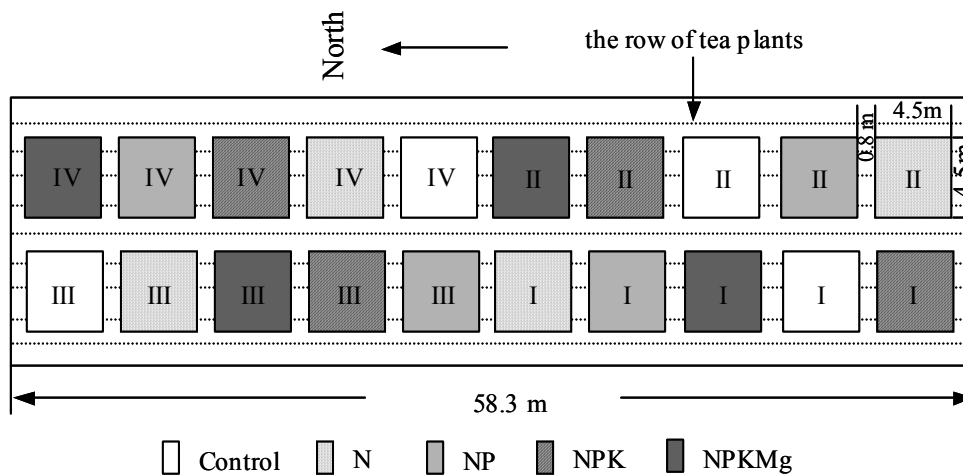


Fig. 4.2 Sketch of experimental field set-up for Biyun in tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China.

Table 4.1 The chemical properties of the soil in the experimental site of Longjing43.

Soil depth (cm)	Total N (%)	Available P* (mg kg <sup>-1</sup> )	Exchangeable K <sup>£</sup> (mg kg <sup>-1</sup> )	Exchangeable Mg <sup>£</sup> (mg kg <sup>-1</sup> )	Available sulphate <sup>§</sup> (mg kg <sup>-1</sup> )	Organic matter (%)	pH <sub>H2O</sub>
0-20	0.08	40.01	53.25	16.00	43.78	2.59	4.16
20-40	0.05	10.32	39.02	18.67	85.44	1.39	4.06

\*: The extractant for P is 0.03 mol L<sup>-1</sup> NH<sub>4</sub>F and 0.025 mol L<sup>-1</sup> HCl;

£: The extractant for K, Mg is 1.0 mol L<sup>-1</sup> NH<sub>4</sub>OAc;

§: The extractant for SO<sub>4</sub><sup>2-</sup> is 0.01 mol L<sup>-1</sup> Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and 2 mol L<sup>-1</sup> HOAc.

Table 4.2 The chemical properties of the soil in the experimental site of Biyun.

Soil depth (cm)	Total N (%)	Available P* (mg kg <sup>-1</sup> )	Exchangeable K <sup>£</sup> (mg kg <sup>-1</sup> )	Exchangeable Mg <sup>£</sup> (mg kg <sup>-1</sup> )	Available sulphate <sup>§</sup> (mg kg <sup>-1</sup> )	Organic matter (%)	pH <sub>H2O</sub>
0-20	0.05	141.00	46.58	17.00	16.55	3.68	3.80
20-40	0.06	64.20	27.78	14.39	34.54	1.87	3.67

\*: The extractant for P is 0.03 mol L<sup>-1</sup> NH<sub>4</sub>F and 0.025 mol L<sup>-1</sup> HCl;

£: The extractant for K, Mg is 1.0 mol L<sup>-1</sup> NH<sub>4</sub>OAc;

§: The extractant for SO<sub>4</sub><sup>2-</sup> is 0.01 mol L<sup>-1</sup> Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and 2 mol L<sup>-1</sup> HOAc.

### 3. Results

#### 3.1. Effects of K and Mg fertilizers on yield and quality elemental contents in flushes and leaves

The yield of Longjing43 in spring of 2002 responded significantly to the fertilizers, while in summer and autumn of 2002, it was not affected by the fertilizers (Table 4.3). The total yield in 2002 was not significantly affected by fertilization. There was an increasing tendency in yield with the increase in fertilizer types. In 2003, the yield in three seasons was significantly increased by the application of fertilizers, especially by P, K and Mg fertilizers (Table 4.3). The yield with NPK treatment in spring and summer of 2003 was higher than that in the other treatments in the same season and the total yield in 2003 showed the same tendency. The total yield with NPK in 2003 was increased by 13% compared to NP, while the yield with NPK was 5% higher than that with NPKMg in 2003.

Compared to 2002, the effect of different fertilizers on yield was significant in 2003 (Table 4.3). Compared to Longjing43, Biyun showed much higher yield in 2002, but the fertilization had no effects on the yield in seasons. In 2003, different treatments did still not show any significant effect on yield in seasons and years, though the increasing tendency existed in 2003 from control to NPKMg treatment (Table 4.4).

Table 4.3 The responses of yield of Longjing43 to different fertilizers (unit: kg FW ha<sup>-1</sup>).

Year	Season	Control	N	NP	NPK	NPKMg
2002	Spring	2708ab <sup>§</sup>	2874b	2605a	2777ab	2659ab
	Summer	3260a	3199a	3729a	3855a	4084a
	Autumn	179a	162a	171a	178a	184a
	Total	6148a	6955a	6505a	6809a	6927a
2003	Spring	1022a	1270b	1167ab	1224b	1207b
	Summer	2865a	3552ab	3578ab	4180b	3880b
	Autumn	82a	117ab	148b	126ab	158b
	Total	3968a	4939b	4893b	5530b	5245b

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

Table 4.4 The responses of yield of Biyun to different fertilizers (unit: kg FW ha<sup>-1</sup>).

Year	Season	Control	N	NP	NPK	NPKMg
2002	Spring	2803a <sup>§</sup>	3063a	2697a	2983a	2850a
	Summer	4040a	4168a	3763a	4182a	4484a
	Autumn	287a	368a	361a	392a	361a
	Total	7129a	7599a	6821a	7558a	7696a
2003	Spring	884a	1001a	923a	939a	912a
	Summer	2344a	2292a	2899a	2934a	3125a
	Autumn	244a	347a	215a	391a	361a
	Total	3472a	3640a	4037a	4264a	4398a

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

Table 4.5 The effect of different fertilizers on the contents of amino acids (AA, %), tea polyphenols (TP, %), the TP/AA ratio (the ratio of tea polyphenols to amino acids) and the content of caffeine (Caf., %) in the tea flushes of Longjing43 in 2002 and 2003.

Year	Quality	Season	Control	N	NP	NPK	NPKMg
2002	AA	Spring	2.43a <sup>§</sup> C <sup>£</sup>	2.58aC	2.26aC	2.46aC	2.35aC
		Summer	1.21aB	1.18aB	1.29aB	1.34aB	1.18aB
		Autumn	0.77aA	0.78aA	0.82aA	0.85aA	0.77aA
	TP	Spring	30.21aB	33.70bC	30.84abB	29.58aB	29.25aB
		Summer	25.02aA	26.39aA	24.02aA	24.44aA	24.65aAB
		Autumn	31.08aB	30.71aB	31.75aB	30.64aB	31.38a
	TP/AA	Spring	9.26aA	9.38aA	9.57aA	9.33aA	9.08aA
		Summer	10.53aB	10.40aB	10.02aB	10.12aB	10.28aB
		Autumn	9.39aA	9.96aA	9.76aA	9.55aA	9.99aA
	Caf.	Spring	3.27aB	3.60aB	3.23aB	3.17aB	3.22aB
		Summer	2.38aA	2.53aA	2.40aA	2.42aA	2.40aA
		Autumn	3.31aB	3.09aB	3.28aB	3.21aB	3.16aB
2003	AA	Spring	2.30aC	2.96bB	3.18bcB	3.16bcB	3.26cB
		Summer	1.14aA	1.66bA	1.74bA	1.71bA	1.71bA
		Autumn	1.32aB	1.71bA	1.77bA	1.80bA	1.82bA
	TP	Spring	31.80bA	29.67abA	29.75abA	28.62aA	28.88aA
		Summer	35.83bC	31.94aB	30.49aA	30.64aA	31.26aB
		Autumn	34.06bB	30.99aAB	29.91aA	30.53aA	30.90aB
	TP/AA	Spring	13.90bA	10.10aA	9.40aA	9.12aA	8.87aA
		Summer	31.49bC	19.33aB	17.59aB	18.09aB	18.29aB
		Autumn	26.02bB	18.12aB	17.06aB	17.12aB	17.14aB
	Caf.	Spring	3.26aA	3.38aAB	3.42aB	3.36aAB	3.39aAB
		Summer	3.25aA	3.50bB	3.53bB	3.52bB	3.49bB
		Autumn	3.10aA	3.26aA	3.16aA	3.22aA	3.25aA

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

<sup>£</sup>: numbers followed by same capital letters indicate that the treatment means between three seasons within one treatment are not significantly different at  $p < 0.05$  ( $n=4$ ).



Table 4.6 The responses of amino acid components to different fertilizers for Longjing43 in the spring of 2003 (unit:  $\mu\text{mol g}^{-1}$  DM).

Component	Control	N	NP	NPK	NPKMg
Asp	12.09a <sup>§</sup>	15.22ab	16.65b	14.63ab	13.84ab
Asn	0.75a	1.20ab	1.21ab	1.56b	1.32ab
Glu	14.45a	19.53a	19.71a	25.48a	23.86a
Gln	24.21a	35.85ab	36.68ab	43.73b	40.18ab
Theanin	65.97a	92.02b	96.58b	96.62b	93.03b
Ala	5.79a	7.30a	7.40a	8.68a	8.17a
Cys	1.15a	1.29a	1.21a	1.45a	1.40a
Tyr	0.41a	0.46a	0.49a	0.58a	0.51a
Phe	0.38a	0.48a	0.46a	0.49a	0.47a
GABA	8.92a	11.89a	10.82a	12.81a	12.43a
His	0.82a	1.08ab	1.16ab	1.27b	1.15ab
Arg	2.87a	6.78ab	8.15b	9.08b	8.31b
Total	150.95a	208.42ab	216.08ab	230.89b	219.88ab

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

In 2002, compared to other fertilizers, the application of K showed a tendency to increase the content of amino acids in spring. Generally, N, P, K and Mg fertilization had no significant effect on the quality-determined constituents of amino acids, tea polyphenols and caffeine of Longjing43 except some change with time (Table 4.5). However, in 2003, the fertilization significantly increased the contents of amino acids, decreased the contents of tea polyphenols and the TP/AA ratio (the ratio of tea polyphenols to amino acids) compared to control. Considering NP as control, K had no positive effect on TP content and TP/AA ratio in summer and autumn. Caffeine was increased only in summer by the fertilization. In both years, the application of P and K exhibited stronger effects on decreasing TP/AA ratio than the other treatments. The application of Mg slightly inhibited the effect of NPK in this aspect. All the quality-determined constituents were significantly affected by seasons except caffeine, especially in 2003 (Table 4.5). The individual amino

acids were analysed in spring of 2003 (Table 4.6). Compared to control, every individual amino acid was increased by N, P, K and Mg applied, especially arginine that showed much stronger responses to N than any other amino acid. Among the individual amino acids, aspartic acid, asparagine, glutamine, theanine, histidine and arginine were significantly increased by the application of N, P, K and Mg fertilizers. But considering the P supplemented treatment as control, the amino acid contents except aspartic acid were all increased by K and Mg fertilization. The application of Mg decreased the contents of these amino acids. Total amino acids were decreased by 5% after the application of Mg fertilizer. NPKMg also decreased the individual amino acid contents compared to NPK.

Amino acids in tea flushes of Biyun in the spring of 2002 (Table 4.7) significantly responded to fertilization. The application of P, K and Mg decreased the content of amino acids compared to N alone, especially for the treatment with NPKMg that significantly decreased the content of amino acids. The application of K and Mg fertilizers together showed stronger effects on decreasing TP/AA ratio than that of single K fertilizer in summer. But the other quality constituents showed no significantly positive response to fertilization. In 2003, the application of N, P, K and Mg fertilizers increased the content of amino acids in spring. Moreover, the effect of P, K and Mg on increasing the content of amino acids was more significant than that in spring of 2002, especially the K application. Similarly to 2002, the Mg application inhibited the effects of K on improving quality. TP/AA ratio was decreased by the application of N, P, K and Mg. But the effect was not significant. Different seasons had significant effects on their contents.

Table 4.7 The effect of different fertilizers on the contents of amino acids (AA, %), tea polyphenols (TP, %), the TP/AA ratio (the ratio of tea polyphenols to amino acids) and the content of caffeine (Caf., %) in the tea flushes of Biyun in 2002 and 2003.

Year	Quality	Season	Control	N	NP	NPK	NPKMg
2002	AA	Spring	2.50a <sup>§</sup> C <sup>£</sup>	3.44cC	3.11bcC	3.02bcC	2.87abC
		Summer	1.33aB	1.64aB	1.78aB	1.42aB	1.30aB
		Autumn	0.76aA	0.78aA	0.87aA	0.85aA	0.82aA
	TP	Spring	30.41aA	31.66aA	29.23aA	29.01aA	28.96aA
		Summer	31.74aA	32.87aA	28.49aA	29.70aA	27.73aA
		Autumn	31.06aA	28.72aA	29.18aA	29.30aA	29.85aA
	TP/AA	Spring	9.33aA	8.79aA	8.98aA	8.84aA	9.06aA
		Summer	10.64bB	10.88bB	9.81aB	9.77aB	9.53aA
		Autumn	9.51bA	8.70abA	8.43aA	8.62abA	9.04abA
	Caf.	Spring	3.26aA	3.60aB	3.26aB	3.29aA	3.20aA
		Summer	3.00aA	3.04aA	2.91aA	3.06aA	2.91aA
		Autumn	3.27aA	3.30aA	3.47aB	3.40aA	3.31aA
2003	AA	Spring	3.00aB	3.35abB	3.45bB	3.50bB	3.41bB
		Summer	1.52aA	1.63aA	1.65aA	1.75aA	1.73aA
		Autumn	1.74aA	1.88aA	1.85aA	2.05aA	2.01aA
	TP	Spring	27.79aA	26.87aA	25.91aA	26.73aA	26.59aA
		Summer	35.24aB	34.93aC	33.66aB	34.04aB	33.24aB
		Autumn	34.71aB	32.90aB	33.77aB	32.63aB	33.34aB
	TP/AA	Spring	9.33aA	8.07aA	7.52aA	7.65aA	7.85aA
		Summer	23.50aC	21.72aC	21.11aB	19.64aC	19.31aB
		Autumn	19.96aB	17.70aB	18.80aB	15.99aB	16.61aB
	Caf.	Spring	3.49aA	3.47aA	3.46aA	3.45aA	3.47aA
		Summer	4.19aB	4.25aB	4.23aB	4.28aB	3.81aA
		Autumn	3.59aAB	4.03aAB	4.08aB	4.09aB	4.10aA

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  (n=4).

<sup>£</sup>: numbers followed by same capital letters indicate that the treatment means between three seasons within one treatment are not significantly different at  $p < 0.05$  (n=4).

Table 4.8 The effect of different fertilizers on the contents of total ascorbic acid (tAsA,  $\mu\text{mol g}^{-1}$  FW), ascorbic acid (AsA,  $\mu\text{mol g}^{-1}$  FW) and dehydroascorbic acid (DAsA,  $\mu\text{mol g}^{-1}$  FW) in the tea flushes and the chlorophyll content (SPAD) of the fourth fully expanded leaf of Longjing43 in 2003.

Quality	Seasons	Control	N	NP	NPK	NPKMg
tAsA	Spring	314.65a <sup>§</sup> A <sup>£</sup>	239.93aA	194.28aA	201.00aA	208.20aA
	Summer	398.35aB	377.08abB	386.90abB	316.63aB	344.30abB
	Autumn	524.85aC	495.70aC	485.83aC	465.85aC	474.78aC
AsA	Spring	194.70aA	203.70aA	168.93aA	165.33aA	177.23aA
	Summer	297.03aB	288.48aB	309.33aB	258.15aB	274.90aB
	Autumn	452.88aC	418.68aC	408.03aC	396.63aC	398.70aC
DAsA	Spring	19.95aA	36.23aA	25.33aA	35.73aA	30.98aA
	Summer	101.33aB	88.60aB	77.55aB	58.50aA	69.38aA
	Autumn	71.95aB	77.03aAB	77.80aB	69.23aA	76.08aA
SPAD	Spring	69.60aA	73.83bA	75.65bcA	75.10bcA	77.35cA
	Summer	64.10aA	74.60bA	76.75bA	77.05bA	77.68bA
	Autumn	-	-	-	-	-

<sup>§</sup>: numbers followed by same small letters indicate the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

<sup>£</sup>: numbers followed by same capital letters indicate the treatment means over the three seasons within one treatment are not significantly different at  $p < 0.05$  ( $n=4$ ).

The contents of total ascorbic acid, ascorbic acid and dehydroascorbic acid in tea flushes of Longjing43 and Biyun were not significantly affected by the fertilization but by seasons. The AsA/DAsA ratio (ratio of ascorbic acid to dehydroascorbic acid) of Longjing43 were on average 6.5, 3.7 and 5.6 in spring, summer and autumn, respectively (Table 4.8). On average, the NPK application could increase AsA/DAsA ratio of Longjing43 from 2.9 (control) to 4.4 in summer (Table 4.8), but that of Biyun were 3.6, 4.6 and 2.2 in spring, summer and autumn, respectively. The NPK application could increase AsA/DAsA ratio of Biyun from 2.1 (control) to 3.8 on average in autumn (Table 4.9). So K application increased the content of vitamin C in reduced form compared to the other treatments. The highest vitamin C content of Longjing43 showed in autumn followed by that in summer, the lowest content was in spring (Table 4.8); while the vitamin C content of Biyun followed the sequence from high to low: summer, autumn and spring (Table 4.9). The application of N could increase the SPAD values for both varieties significantly, but P, K

and Mg fertilizers had no significant effects on SPAD values, compared to NPK, SPAD only showed a increased tendency in NPKMg in both varieties (Table 4.8, Table 4.9).

Table 4.9 The effect of different fertilizers on the contents of total ascorbic acid (tAsA,  $\mu\text{mol g}^{-1}$  FW), ascorbic acid (AsA,  $\mu\text{mol g}^{-1}$  FW ) and dehydroascorbic acid (DAsA,  $\mu\text{mol g}^{-1}$  FW) the chlorophyll content (SPAD) of the fourth fully expanded leaf in the tea flushes of Biyun in 2003.

Quality	Seasons	Control	N	NP	NPK	NPKMg
tAsA	Spring	325.10a§A£	287.75aA	309.60aA	293.53aA	317.65aA
	Summer	490.88aB	441.85aB	462.03aB	456.97aB	478.45aB
	Autumn	469.05aB	428.18aB	464.08aB	459.85aB	458.38aB
AsA	Spring	235.40aA	239.83aA	244.95aA	233.30aA	228.10aA
	Summer	411.35aC	370.68aB	367.33aB	368.30aC	392.40aC
	Autumn	315.58aB	272.63aA	292.53aA	302.28aB	288.00aB
DAsA	Spring	89.70aA	47.90aA	64.70aA	60.25aA	89.55aA
	Summer	79.53aA	71.20aA	94.73aA	88.70aA	86.05aA
	Autumn	153.45aB	155.55aB	171.53aB	157.55aB	170.35aB
SPAD	Spring	64.98aA	67.03abA	68.60abA	71.10bA	70.98bA
	Summer	62.18aA	70.68bB	72.83bcB	73.92bcA	76.58cB
	Autumn	-	-	-	-	-

§: numbers followed by same small letters indicate the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

£: numbers followed by same capital letters indicate the treatment means over the three seasons within one treatment are not significantly different at  $p < 0.05$  ( $n=4$ ).

### 3.2. Effect of K and Mg on mineral nutrient content

Table 4.10 showed that the mineral nutrient contents in tea flushes of Longjing43 in 2002 were not significantly affected by the N, P, K and Mg fertilizers. However, the total contents of N, P, K, Mg and Ca in flushes were significantly affected by seasons, especially for total contents of N, P, K and Mg. In 2003, for Longjing43, only total contents of N and P in tea flushes in summer and autumn showed a positive response to fertilization. There was a tendency that the application of K fertilizer could promote the uptake of N and P in spring and summer of 2003. Total Ca content in autumn was significantly reduced by the fertilization. Total contents of K, Mg and Ca in tea flushes were not significantly affected by N, P, K and Mg fertilizers. Comparison of three seasons

within each treatment indicated that total contents of N, P and K in flushes were significantly reduced with time. Total Ca content increased with the time going on, while total Mg content was hardly affected by seasons. Total contents of K and Mg in flushes in 2003 were much lower than those in 2002, especially for K, almost half of those in 2002. Contrast to total K content, total Ca content in 2003 was as high as that in 2002 and was not affected by seasons (Table 4.10).

For Biyun, total content of P in flushes was significantly increased by the application of P, K and Mg compared to the control in summer, but also by N alone in 2002 (Table 4.11). The total P content in summer flushes with NP was even slightly lower than that with N alone, despite reduced yield (Table 4.4). Generally the total P content in summer tea flushes was not significantly different to that in spring. The same holds true for Longjing43, all the elemental contents were significantly affected by seasons (Table 4.11). However, in 2003, no effect of fertilization on the total content of mineral nutrients was observed. All the mineral nutrient contents decreased significantly with time. The highest total P content was still in spring. The total K content decreased with time and its content in three seasons were also much lower than those in 2002 (Table 4.11), which exhibited the same tendency with the variety of Longjing43 in 2003 (Table 4.10).

Total contents of P, K, Mg and Ca in the mature leaves in the early spring of 2003 (Table 4.12) indicated that P and Ca contents in the mature leaves were high, but K and Mg contents were quite low for both Longjing43 and Biyun. K and Mg had no effect on the total contents of P, K, Mg and Ca in the mature leaves of Longjing43. For Biyun, the application of K fertilizer could slightly increase the total content of K compared with that in NP. Although the effect was not significant, the tendency existed in Biyun.

Table 4.10 The effect of different fertilizers on the total content of N, P, K, Mg and Ca (%) in the tea flushes of Longjing43 in 2002 and 2003.

Year	Element	Season	Control	N	NP	NPK	NPKMg	
2002	N	Spring	5.49aB	5.69aB	5.64aB	5.55aB	6.33aB	
		Summer	4.07aA	4.16aA	4.27aA	4.27aA	4.31aA	
		Autumn	3.96aA	4.03aA	4.15aA	4.21aA	4.23aA	
	P	Spring	0.69a§C£	0.69aC	0.72aC	0.71aC	0.71aC	
		Summer	0.59aB	0.61aB	0.62aB	0.63aB	0.63aB	
		Autumn	0.38aA	0.42aA	0.42aA	0.42aA	0.41aA	
	K	Spring	1.30aA	1.28aA	1.30aA	1.31aA	1.31aA	
		Summer	1.57aC	1.57aC	1.57aC	1.58aC	1.58aC	
		Autumn	1.37aB	1.37aB	1.39aB	1.39aB	1.39aB	
	Mg	Spring	0.10aA	0.10aA	0.10aA	0.10aA	0.10aA	
		Summer	0.14aC	0.15aC	0.15aC	0.15aC	0.15aC	
		Autumn	0.11aB	0.11aB	0.11aB	0.11aB	0.11aB	
	Ca	Spring	0.10aA	0.09aA	0.09aA	0.09aA	0.09aA	
		Summer	0.11aB	0.11aB	0.10aB	0.11aB	0.10aB	
		Autumn	0.11aB	0.11aB	0.11aB	0.10aB	0.10aB	
	2003	N	Spring	5.72aC	5.82abC	6.06abC	6.24bC	6.12bC
			Summer	4.12aA	4.61bA	4.67bA	4.70bA	4.61bA
			Autumn	4.41aB	4.87bB	4.92bB	4.91bB	4.98bB
P		Spring	0.81aC	0.81aC	0.80aC	0.82aC	0.82aC	
		Summer	0.51aB	0.57bB	0.57bB	0.58bB	0.57bB	
		Autumn	0.42aA	0.46abA	0.48bA	0.48bA	0.49bA	
K		Spring	0.74aC	0.74aC	0.73aC	0.75aC	0.75aC	
		Summer	0.68aB	0.68aB	0.68aB	0.69aB	0.69aB	
		Autumn	0.56aA	0.57aA	0.58aA	0.57aA	0.58aA	
Mg		Spring	0.10aA	0.10aA	0.09aA	0.09aA	0.10aA	
		Summer	0.09aA	0.09aA	0.09aA	0.09aA	0.09aA	
		Autumn	0.09aA	0.09aA	0.09aA	0.09aA	0.09aA	
Ca		Spring	0.10aA	0.10aA	0.09aA	0.10aA	0.10aA	
		Summer	0.11aB	0.11aB	0.11aB	0.11aB	0.11aB	
		Autumn	0.12bB	0.11aB	0.11aB	0.11aB	0.11aB	

§: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

£: numbers followed by same capital letters indicate that the treatment means between three seasons within one treatment are not significantly different at  $p < 0.05$  ( $n=4$ ).

Table 4.11 The effect of different fertilizers on the total content of N, P, K, Mg and Ca (%) in the tea flushes of Biyun in 2002 and 2003.

Year	Element	Season	Control	N	NP	NPK	NPKMg	
2002	N	Spring	5.83abC	5.07aC	6.14bC	6.06bC	5.80abC	
		Summer	4.35aB	4.64aB	4.55aB	4.59aB	4.53aB	
		Autumn	3.97aA	4.23aA	4.20aA	4.27aA	4.15aA	
	P	Spring	0.70a <sup>§</sup> B <sup>£</sup>	0.70aB	0.71aB	0.71aB	0.71aB	
		Summer	0.69aB	0.77bB	0.74bB	0.76bB	0.75bB	
		Autumn	0.41aA	0.43aA	0.42aA	0.44aA	0.42aA	
	K	Spring	1.15aA	1.14aA	1.12aA	1.15aA	1.16aA	
		Summer	1.56aC	1.58aC	1.54aC	1.57aC	1.57aC	
		Autumn	1.29aB	1.30aB	1.28aB	1.33aB	1.32aB	
	Mg	Spring	0.10aA	0.10aA	0.09aA	0.10aA	0.10aA	
		Summer	0.15aC	0.15aC	0.15aC	0.15aC	0.15aC	
		Autumn	0.12aB	0.12aB	0.11aB	0.12aB	0.12aB	
	Ca	Spring	0.09aA	0.09aA	0.09aA	0.09aA	0.09aA	
		Summer	0.11aB	0.11aB	0.11aB	0.11aB	0.11aB	
		Autumn	0.10aB	0.10aB	0.10aB	0.10aB	0.10aB	
	2003	N	Spring	6.36aC	6.40aC	6.53aC	6.50aC	6.32aC
			Summer	4.32aA	4.43aA	4.67aA	4.59aA	4.52aA
			Autumn	5.45aB	5.51aB	5.43aB	5.61aB	5.56aB
P		Spring	0.77aC	0.79aC	0.79aB	0.78aC	0.79aC	
		Summer	0.45aA	0.45aA	0.49aA	0.48aA	0.46aA	
		Autumn	0.53aB	0.55aB	0.54aA	0.57aB	0.57aB	
K		Spring	0.71aC	0.71aC	0.70aC	0.71aC	0.72aC	
		Summer	0.59aB	0.59aB	0.62aB	0.62aB	0.61aB	
		Autumn	0.55aA	0.55aA	0.55aA	0.56aA	0.56aA	
Mg		Spring	0.09aA	0.09aA	0.09aA	0.09aA	0.09aA	
		Summer	0.09aA	0.09aA	0.09aA	0.09aA	0.09aA	
		Autumn	0.09aA	0.09aA	0.09aA	0.10aA	0.10aA	
Ca		Spring	0.10aA	0.09aA	0.10aA	0.10aA	0.10aA	
		Summer	0.11aB	0.11aB	0.11aB	0.11aB	0.11aB	
		Autumn	0.10aA	0.09aA	0.10aA	0.09aA	0.09aA	

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

<sup>£</sup>: numbers followed by same capital letters indicate that the treatment means between three seasons within one treatment are not significantly different at  $p < 0.05$  ( $n=4$ ).



Table 4.12 The total contents of P, K, Mg and Ca (%) in mature leaves that were on the brown stems and grew up from the former year in Longjing43 and Biyun sites before the fertilization in the early spring of 2003.

Variety	Element	Control	N	NP	NPK	NPKMg
Longjing43	P	0.19a <sup>§</sup>	0.19a	0.19a	0.20a	0.19a
	K	0.57a	0.58a	0.60a	0.62a	0.61a
	Mg	0.06a	0.06a	0.06a	0.05a	0.06a
	Ca	0.12a	0.12a	0.12a	0.12a	0.12a
Biyun	P	0.17a	0.17a	0.17a	0.17a	0.17a
	K	0.53a	0.51a	0.49a	0.56a	0.55a
	Mg	0.08a	0.07a	0.08a	0.07a	0.07a
	Ca	0.12a	0.12a	0.12a	0.12a	0.12a

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one season are not significantly different at  $p < 0.05$  ( $n=4$ ).

### 3.3. Soil chemical properties

The application of K and Mg fertilizers only increased the soil exchangeable K and Mg contents in soil in Longjing43 experimental field in 2003 (Table 4.13). Compared to 2002, in 2003, the contents of soil available P had been increased by 71% in 0-20 cm soil and 166% in 20-40 cm soil. The exchangeable K content was increased by 44% and 87% in 0-20 cm and 20-40 cm soil, respectively. The exchangeable Mg contents were increased by 65% and 87% in 0-20 cm and 20-40 cm soil, respectively (Table 4.13). Soil pH in 2003 was decreased by the application of fertilizers, especially compared to that in 2002. In Biyun site (Table 4.14), the fertilization almost had no effect on the soil mineral element contents and soil pH in 2002. Compared to those in 2002, in 2003, soil available P was increased by 97% and 112% in 0-20 cm and 20-40 cm soil on average, respectively. Exchangeable K was increased by 99% and 122% on average in both soil depth, and exchangeable Mg was increased by 80% and 298% in 0-20 cm and 20-40 cm soil depth. Soil pH in 0-20 cm and 20-40 cm soil depth was increased by 14% and 12%, respectively. Compared to the content of soil organic matter (Table 4.2), the content of organic matter in soil at the end of 2003 had also been increased slightly after the fertilization for two

years, though the contents between treatments did not differ significantly. Compared to 2002, the soil available P, exchangeable K, Mg and Ca were increased by 106%, 42%, 88% and 39% and pH was decreased by 3.7% in control of both sites in 2003 on average, respectively.

The exchangeable K, Mg and Ca content in soil in the early spring of 2003 (Table 4.15) showed similar levels to those in the late autumn of 2002 in Longjing43 site, but the application of K significantly increased the exchangeable K content in Biyun site and the exchangeable K content was generally lower than those in the late autumn of 2002. Compared to those in the early spring (Table 4.15), the exchangeable K, Mg and Ca contents in the late autumn of 2003 were highly increased in both sites (Table 4.13 and Table 4.14).

Table 4.13 The contents of soil available P (Avail. P, mg kg<sup>-1</sup>), soil exchangeable K, Mg, Ca (Exchang. K, Mg, Ca, mg kg<sup>-1</sup>) in 0-20 cm and 20-40 cm depth soil and soil organic matter (Org. M., %) and soil pH<sub>H2O</sub> for Longjing43 site in the late autumn of 2002 and 2003.

Year	Elemental content	Depth (cm)	Control	N	NP	NPK	NPKMg	
2002	Avail. P	0-20	67.22a <sup>§</sup> B <sup>£</sup>	75.00aB	109.01aB	113.84aB	138.33aB	
		20-40	34.39aA	76.04aA	46.80aA	52.39aA	47.52aA	
	Exchang. K	0-20	66.76aB	67.98aB	68.08aB	73.00aB	68.62aB	
		20-40	48.41aA	49.77aA	47.95aA	55.87aA	40.90aA	
	Exchang. Mg	0-20	11.43aA	13.28aA	12.23aA	10.94aA	16.41aA	
		20-40	11.08aA	14.97aA	11.73aA	11.16aA	13.03aA	
	Exchang. Ca	0-20	113.14aA	111.17aA	128.25aA	110.48aA	128.73aA	
		20-40	105.07aA	111.22aA	110.05aA	101.51aA	92.49aA	
	pH <sub>H2O</sub>	0-20	4.28aA	4.01aA	4.23aA	3.95aA	4.12aA	
		20-40	4.31aA	4.17aA	4.20aA	4.08aA	4.16aA	
	2003	Avail. P	0-20	104.72aA	139.85aA	203.28aA	214.80aA	201.70aA
			20-40	88.68aA	101.32aA	189.64aA	139.68aA	165.70aA
Exchang. K		0-20	87.42abA	82.60aA	83.31aA	131.28bA	110.56abA	
		20-40	74.23aA	69.55aA	72.46aA	122.28bA	102.66abA	
Exchang. Mg		0-20	22.91abA	18.90aA	20.10abA	20.98abA	26.53bA	
		20-40	19.55aA	21.02aA	21.96aA	22.57aA	30.54bA	
Exchang. Ca		0-20	169.75aA	101.22aA	110.05aA	138.22aA	134.70aA	
		20-40	134.66aA	209.13aA	149.89aA	134.87aA	132.00aA	
Org.M.		0-20	1.84aA	1.95aB	2.09aA	2.31aB	1.65aA	
		20-40	1.36aA	1.44aA	1.69aA	1.77aA	1.63aA	
pH <sub>H2O</sub>		0-20	4.12aA	3.72aA	3.79aA	3.89aA	3.93aA	
		20-40	4.15bA	3.96aA	3.90aA	3.95aA	3.69aA	

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means within one depth are not significantly different at  $p < 0.05$  (n=4).

<sup>£</sup>: numbers followed by same capital letters indicate that the treatment means between two depth within one treatment are not significantly different at  $p < 0.05$  (n=4).

Table 4.14 The contents of soil available P (mg kg<sup>-1</sup>), soil exchangeable K, Mg, Ca (mg kg<sup>-1</sup>) in 0-20 cm and 20-40 cm depth soil and soil organic matter (Org.M., %) and soil pH<sub>H2O</sub> for Biyun site in the late autumn of 2002 and 2003.

Year	Elemental content	Depth (cm)	CK	N	NP	NPK	NPKMg	
2002	Avail. P	0-20	255.18a <sup>§</sup> B <sup>£</sup>	129.24aA	156.98aA	166.78aA	140.83aA	
		20-40	130.13aA	116.83aA	93.76aA	107.60aA	100.02aA	
	Exchang. K	0-20	44.84aB	37.76aA	45.97a	41.21aA	42.52aA	
		20-40	23.02aA	25.98aA	25.61aA	23.01aA	44.64aA	
	Exchang. Mg	0-20	22.83bB	14.02aA	14.67aB	12.40aA	12.45aA	
		20-40	11.95aA	10.95aA	9.19aA	7.09aA	10.58aA	
	Exchang. Ca	0-20	184.20bB	122.58aAb	155.83abB	114.70aA	120.75abA	
		20-40	98.67aA	98.64aA	83.10aA	71.38aA	113.00aA	
	pH <sub>H2O</sub>	0-20	4.03aA	3.73aA	4.02aA	3.84aA	3.79aA	
		20-40	4.06aA	3.77aA	3.40aA	3.79aA	4.20aA	
	2003	Avail. P	0-20	302.05aA	263.58aA	340.25aB	369.08aA	399.75aA
			20-40	200.18aA	211.48aA	185.45aA	263.43aA	304.00aA
Exchang. K		0-20	66.48aA	61.40aA	80.12aA	86.34abA	128.50bA	
		20-40	52.05aA	35.30aA	50.66aA	59.15aA	118.37bA	
Exchang. Mg		0-20	45.48aA	32.29aA	45.41aA	30.54aA	60.19aA	
		20-40	43.11aA	28.34aA	35.11aA	30.79aA	60.62aA	
Exchang. Ca		0-20	310.85aA	213.21aA	395.39aA	171.63aA	316.78aA	
		20-40	309.00aA	157.61aA	174.90aA	130.98aA	273.43aA	
Org.M.		0-20	4.22aB	3.87aB	4.65aB	3.95aB	3.98aB	
		20-40	2.24aA	1.95aA	2.32aA	1.77aA	2.66aA	
pH <sub>H2O</sub>		0-20	3.49aA	3.28aA	3.33aA	3.60aA	3.39aA	
		20-40	3.68aA	3.34aA	3.19aA	3.38aA	3.51aA	

<sup>§</sup>: numbers followed by same small letters indicate the treatment means within one depth are not significantly different at  $p < 0.05$  (n=4).

<sup>£</sup>: numbers followed by same capital letters indicate the treatment means over two depth within one treatment are not significantly different at  $p < 0.05$  (n=4).

Table 4.15 The contents of soil exchangeable K, Mg and Ca (Exchang. K, Mg and Ca) in 0-20 cm and 20-40 cm soil depth in Longjing43 and Biyun sites before the fertilization in the early spring of 2003.

Variety	Elemental content	Depth (cm)	Control	N	NP	NPK	NPKMg
Longjing43	Exchang. K	0-20	53.28a <sup>§</sup> B <sup>£</sup>	51.08aB	52.63aB	72.24aB	56.52aB
		20-40	33.00aA	34.05aA	34.23aA	52.81aA	42.51aA
	Exchang. Mg	0-20	10.91aA	10.74aA	8.79aA	11.70aA	14.48aA
		20-40	11.84aA	10.45aA	7.85aA	9.02aA	12.65aA
	Exchang. Ca	0-20	109.46aA	111.60aA	86.41aA	105.68aA	103.37aA
		20-40	94.08aA	109.88aA	76.70aA	89.68aA	97.67aA
Biyun	Exchang. K	0-20	30.70aA	31.86aA	30.18aA	69.45bB	61.94bB
		20-40	20.55aA	21.12aA	18.30aA	29.91abA	36.91bA
	Exchang. Mg	0-20	20.15bA	12.43aA	12.37aA	20.64bA	17.85abA
		20-40	15.67abA	12.23aA	10.88aA	13.43aA	14.44abA
	Exchang. Ca	0-20	138.00bA	102.37Aa	90.52aA	134.44bA	106.75aA
		20-40	86.72aA	106.20aA	81.37aA	150.37bA	86.52aA

<sup>§</sup>: numbers followed by same small letters indicate the treatment means within one depth are not significantly different at  $p < 0.05$  ( $n=4$ ).

<sup>£</sup>: numbers followed by same capital letters indicate the treatment means over two depth within one treatment are not significantly different at  $p < 0.05$  ( $n=4$ ).

#### 4. Discussion

Nutrient deficiency and unbalance in soils lead to low yield and poor quality of tea. The empirical threshold for soil exchangeable K and Mg are 80 mg kg<sup>-1</sup> (He and He, 1993) and 40 mg kg<sup>-1</sup> (Tea Research Institute, China, personal communication) in China, respectively. The contents of exchangeable K and Mg in both sites were not sufficient at the beginning of the experiments (Table 4.1, Table 4.2), which caused the essentiality to apply potassium and magnesium fertilizers. The yields of Longjing43 and Biyun were increased after the application of fertilizer K and Mg, especially for Longjing43, its yield was significantly increased by K and Mg fertilization in three seasons in 2003 (Table 4.3). These results were consistent with the studies of Gohain (1994) and Wu (1994b). The significant differences between both years for two varieties were caused by the different plucking frequency except the differences between varieties.

Leaf nutrient contents indicated that total content of P in tea flushes was sufficient (usually the total content of P in plants is 0.3-0.5%, Marschner, 1995), the total P content decreased with the time. One of the reasons is that the absorbed P was primarily used to meet reproductive growth, the other might be that it is difficult for the available P in soil solution to diffuse to the roots and inhibited the uptake of P by tea plants, especially in drought conditions in 2003 (Table 1.2). The empirical total K and Mg contents in flushes were 1.2-2.0% (Krishnapillai and Ediriweera, 1986; Verma, 1997) and 0.12-0.3% (Hettiarachchi et al., 1997), respectively. The total K and Mg contents in mature leaves were 1.4-2.0% (Othieno, 1983; Krishnapillai and Ediriweera, 1986;) and 0.1% (Othieno, 1983), respectively. According to these standards, the total contents of K and Mg were sufficient for both varieties only in 2002 and the general magnesium content increased with the application of K and Mg fertilizers and with time. However, it was evident that the total contents of K and Mg in the tea flushes of both varieties in 2003 were deficient and that K and Mg in mature leaves for both varieties were not sufficient as well (Table 4.12). The deficiency of K and Mg in mature leaves in the early spring of 2003 implied that the deficiency of K and Mg in tea plants happened between 2002 and 2003. Under the condition that there was no big change of soil exchangeable K and Mg contents in the late autumn of 2002 and the early spring of 2003 (Tables 4.13-4.15), why were the differences between K and Mg contents in the late autumn tea flushes of 2002 and that in the early spring of 2003 so significant (Table 4.10 and Table 4.11)? The possible reasons were described as the following: i) After one-year experiment, K and Mg stored in tea plants had been consumed, K and Mg started to be not sufficient in it. K and Mg contents in mature leaves verified this point. But there was no significant effect of K and Mg on yield (Table 4.3 and Table 4.4) and quality (Table 4.5 and Table 4.7) observed, even in the treatment of NPK and NPKMg, therefore, this hypothesis was impossible. ii) During the time of late autumn-winter-early spring, the fast growth of tea plants diluted K and Mg within tea plants, which resulted in the decrease of K and Mg in mature leaves in the early spring of 2003. Concerning why were the contents of K and Mg in flushes and mature leaves with NPK and NPKMg deficient? Why did not K and Mg have significant effects compared to NP? This is might be caused by the different growth rates in different

treatments. Because it was obviously observed in the field that the growth of tea plants in control was very poor (thin, yellow-green and with less tea flushes), but in the treatments of NPK and NPKMg, the growth of tea plants was very good (strong and dark green), which was one of the reasons caused the differences in yield in Table 4.3 and Table 4.4. The different growth rate resulted in the different dilution for K and Mg in tea plants. Therefore, no significant effect was observed in spring of 2003. iii) Under extreme drought condition, the reduced availability of K and Mg in soil solution restricted the absorption of K and Mg by tea plants. Compared to the late autumn of 2002, the soil exchangeable K and Mg in the early spring of 2003 really showed the decreased tendency (Table 4.15). The strong change of soil mineral elemental contents between 2002 and 2003 could not be explained very well just according to the results in this study. The application of sulphur powder in a large quantity in the treatments except NPKMg caused the decreased soil pH. The decreased soil pH could be responsible a little for that. Maybe the extreme drought condition (Table 1.1 and Table 1.2) or any other factor that was not covered in this study caused the significant increase of soil mineral elemental contents (Table 4.13 and Table 4.14). But compared to the early spring of 2003, soil exchangeable K and Mg in the end of 2003 were also significantly increased, which further indicated that the change of soil mineral elemental contents happened within 2003, though it was difficult to explain. The lower K and Mg contents in mature leaves (Table 4.12) and the high soil elemental contents in the late autumn of 2003 told us that the effect of K and Mg on yield and quality could be expected to be observed. But only the slight yield response (Table 4.3 and Table 4.4) and the individual amino acid responses (Table 4.6) to K and Mg were observed beside the growth responses of tea plants to K and Mg in the field. Compared to NP, glutamic acid, glutamine, GABA, alanine and arginine with NPK were increased 29%, 19%, 18%, 17% and 11%, respectively. This increase was associated with the function of K and the interaction with N in the growth of tea plants. The application of fertilizer K actually increased the exchangeable K content in soil, especially in 2003 in both experimental sites, but the Mg/K ratios were also increased from 0.19, 0.26 to 0.23, 0.27 in both soil depth in Longjing43, respectively and from 0.36, 0.37 to 0.52, 0.67 in both soil depth in Biyun site, respectively (Table 4.13, Table 4.14). Excess of K in the soil

may cause shortage of Mg in the plant and vice-versa (Wettasinghe and Watson, 1980). The increased Mg/K values in one hand resulted in the antagonism of Mg and K uptake by tea plants because of the well-known antagonistic action between K and Mg, which could be reflected by almost all the results of quality in NPK and NPKMg treatments, especially for Biyun. Compared to NPK, NPKMg could not significantly increase the content of amino acids and decrease the content of TP/AA ratio, even decrease the content of amino acids and increase the TP/AA ratio in Biyun (Table 4.5 and Table 4.7). The contents of individual amino acids with NPKMg were all lower than those with NPK (Table 4.6). On the other hand, the increased Mg/K values might result in a reduced risk for Mg deficiency. Because the possibility of Mg deficiency has been reported to be related to the lower Mg/K ratio of the soils. With the comparison of both varieties, in Biyun site in 2003 (Table 4.14), the exchangeable K and Mg contents were increased significantly and reached the empirical critical levels. So its improvement to quality caused by fertilizers K and Mg were not so significant as that of Longjing43 in 2003 (Table 4.5 and Table 4.6).

It was important to note that a magnesium content as low as 0.09% did not impose any limitation on yield. When the total content of Mg in first leaf fell to 0.08%, there was no significant increase with magnesium fertilization and it was also thought that tea plants can tolerate a wide variation of the level of magnesium supply without adverse effects on yield (Willson, 1975a). Magnesium deficiency was often reported to be caused by low pH and excessive calcium in the soil both of which reduced Mg uptake by plants (Obatolu, 1999). In 2003, sulphur powder was applied as sulphur source in the treatments except NPKMg in a large amount caused the acidification of soil, which resulted in the increase of the availability of Ca in soil (Table 4.13 and Table 4.14). The mobility and the intensity of Mg are stronger than those of Ca in soil. In acid soil, the exchangeable Mg content is much lower than that of Ca because of its strong mobility. Moreover, due to antagonism between Ca and Mg, the uptake to Mg by tea plants is affected by Ca (Wu, 1994a). On average, in this study, the exchangeable Ca was increased by 10.51% in 0-20 cm, 46.16% in 20-40 cm in Longjing43 site and 101.68% in 0-20 cm, 125.03% in 20-40 cm in Biyun site, respectively (Table 4.13, Table 4.14). Significant increase of exchangeable Ca in Biyun site (Table 4.14) was one of the reasons why the application of fertilizers containing



K and Mg had a smaller effect on the quality (Table 4.7) compared to that of Longjing43 in 2003 (Table 4.5, Table 4.6). According to plant analysis and soil test for several years (Wu, 1994a), the contents of Ca and Mg and the Ca/Mg value differentiated in various organs of the normal growing tea plants. The mobility of magnesium allows the plant to transfer the element from old leaves to growing ones by long-distance transport of phloem (Marschner, 1995), so Mg preferentially meets the requirement of growing points, that is, the young tissues of tea plants. In the long-distance transport of cations in the xylem, Ca shows a stronger cation-exchange adsorption reaction with cell wall components, which results in its poor mobility in plants (Marschner, 1995). In dry climate, a large amount of Ca was accumulated in cell walls in mature leaves caused by strong transpiration with long-distance transport of xylem. Therefore, Ca usually accumulates in aged tissues. The Ca/Mg value got higher rapidly as the tissues got older. The Ca/Mg value was above 1 in leaves but below 1 in roots (Wu, 1994a). The graded quality of made tea dropped with the increasing of Ca/Mg value in fresh leaves. The Ca/Mg ratios of Longjing43 in 2002 and 2003 were 0.95, 0.70 and 0.96 as well as 0.10, 1.22 and 1.22 in spring, summer and autumn, respectively. The Ca/Mg ratio of Biyun in 2002 and 2003 were 0.95, 0.73 and 0.83 as well as 1.05, 1.22 and 0.95 in spring, summer and autumn, respectively (Table 4.10, Table 4.11). These results indicated that the tea flushes plucked in 2003 were older than those in corresponding seasons in 2002. Moreover, the uptake of Mg and Ca by tea plants from the soil depended on not only their contents but also their balance. Either the deficiency of elements or imbalance of them would decrease the growth and the quality of tea flushes, resulting in the low yield and inferior quality of made tea (Wu, 1994a). This could explain why the effect detected of K and Mg fertilization on yield and quality of Biyun was smaller, especially in 2003 (Table 4.4 and Table 4.7) and it is likely that this was caused by the imbalance of Ca and Mg in soil.

Although the total contents of K and Mg in tea flushes of both varieties in 2003 were not as high as the critical level (Table 4.10 and Table 4.11), the effect to improve the quality, especially for Longjing43, had been illustrated in Tables 4.5-4.7. Although fertilization had no effect on TP and caffeine contents, the application of P, K and Mg fertilizers generally increased the contents of free amino acids, decreased the TP/AA ratio that

showed significantly negative linear regression with green tea quality (Shi and Liu, 1987). But Longjing43 showed more significant responses to fertilization (Table 4.5) and Biyun showed smaller responses to the fertilization in both years (Table 4.7). The reasons mentioned above about the effects of fertilization on the contents of elements in flushes could be used to explain why a smaller effect concerning the improvement of the quality of Biyun was found in the second year. Because it had been reported that significant responses to K occurred in soils of pH from 4.4 to 5.2 (Lin, 1967 in Willson, 1975b). In the site of Biyun, the very low soil pH was one of the main reasons to reduce the effect of K, even Mg fertilizer. From the results in Table 4.7, the conclusion could be made that the application of N, P, K and Mg fertilizers could increase the contents of amino acids, but the application of NPK had more significant effect on improving the quality compared to NPKMg because of the antagonism of K and Mg. Although there were no significant differences between NPK and NPKMg treatments, the values tended to be lower in NPKMg than those in NPK. However, the application of K and Mg together could still increase the content of amino acids compared to other treatments, which is related to their nutrient functions. Because K is linked with almost all the phenomena of plant physiology. Moreover, K can increase the resistance of plants to drought. In the second year, which was dry (meteorological data see Table 1.1 and Table 1.2), though water stress inhibited the diffusion flux rate of nutrients to roots, it is evident that the application of K increased the resistance of tea plants to drought according to this study. Although we could not tell the absorbed K was either primarily used for the growth of tea flushes or primarily to resist drought stress, the application of K fertilizer significantly increased the yield, especially for the yield of Longjing43 in summer of 2003 (Table 4.3 and Table 4.4) and improved the quality especially for Longjing43 in 2003 (Table 4.5-4.7). Ascorbic acid was not significantly affected by the different fertilizers. Fertilization only had the tendency to increase AsA/DAsA ratio of Longjing43 in summer and that of Biyun in autumn. The ratio of AsA/DAsA was affected by the season. The contents of vitamin C in summer and autumn were significantly higher than those in spring for both varieties. The higher vitamin C content in summer and early autumn indicated that the vital protection system of inhibiting the free radical-associated damage may be driven after a hot and high

irradiance summer (meteorological data in Table 1.1 and Table 1.2). The application of K enhanced the antioxidant power of tea plants.

So far, the relationship between K and Mg fertilizers and the quality could not be explained well in this study and the relationship between ascorbic acid and fertilizer K and Mg still needs further study. The decreased soil pH caused by the application of sulphur powder in a large amount was one of the main reasons to result in the big difference of elemental contents in the soil. The complicated change of K and Mg in soil between years brought about a question to us, that is, to study the dynamics of K and Mg mineral nutrients in soil to build up the relationship between K, Mg and yield as well as quality of green tea.



## Chapter 5

**The effect of sulphur nutrition on the growth and quality of green tea (*Camellia sinensis* L.)****Abstract**

36-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of clone Ju Huachun) in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences were used to study the effect of sulphur on yield and quality of green tea, which consisted of two S fertilizer rates (0, 50 kg S ha<sup>-1</sup> a<sup>-1</sup>) and formed a 2×4 completely randomized block design with four replications. Sulphur application could slightly increase the fresh yield, increase the content of free amino acids, total ascorbic acid (tAsA), ascorbic acid (AsA), dehydroascorbic acid (DAsA), decrease the content of tea polyphenols and decrease the ratio of tea polyphenols to free amino acids (TP/AA). Meanwhile, the application of sulphur could slightly decrease the N:S ratio, but the effect was not significant. Moreover, sulphur application could slightly increase the activity of nitrate reductase (NR) and phenylalanine ammonia-lyase (PAL), but decrease the activity of polyphenol oxidase (PPO), which could be used in the explanation to the effect of sulphur on yield and quality of green tea.

**1. Introduction**

Sulphur (S) has had a significant role in the agricultural production growth thus far. It has a variety of vital functions within the plant's biochemistry. It is a major constituent of amino acids, such as cysteine and methionine – the building blocks of proteins. It is also essential in the formation of enzymes, vitamins, such as biotin and thiamine, and a variety of other important compounds in the plant, including chlorophyll (Ceccotti, 1996). The effect of sulphur fertilizers is easily neglected by widely applying the sulphur-containing fertilizers, such as ammonium sulphate (S 24%) and calcium superphosphate (S 12%). When they are applied to soil as nitrogen and phosphorus fertilizers, they improve the sulphur nutrient supply. However, more recently, with the development of high-concentration fertilizers, non-sulphur-containing and low sulphur-containing fertilizers, such as urea, diammonium sulphate and triple superphosphate are produced and being used to meet crop requirements for nitrogen and

phosphorus, by this ammonium sulphate and single superphosphate no longer comprise the large percent of the total nitrogen or phosphorus fertilizer applied, as they have in previous years. This change causes an increasing deficit in sulphur as crop production climbs. Another event has affected worldwide demand for sulphur fertilizer consumption and will become of greater importance in the future. Increased environmental concern has prompted reductions in sulphur dioxide emissions, which in the past, has benefited crop growth in many countries. Sulphur dioxide from the atmosphere finds its way into the plant through the soil, whether through precipitation or dry deposition. The Environmental Law Institute reports that many industrialized countries have made dramatic reductions in their sulphur dioxide emissions over the last several years (Messick et al., 1996). So sulphur application will play a greater role into the 21<sup>st</sup> century.

In China, tea plants mainly grow in the acid soils that are humid and rich in aluminium. These regions, with a warmer climate and high rainfall, are generally accompanied with intense weathering and strong leaching of soil mineral sulphur. Combined with the promotion of intensive agriculture, such as the use of high yielding varieties and the decrease of the incidental application of sulphur-containing fertilizers caused by the development of chemical fertilizer industry, soils are more likely to be deficient in sulphur. Thus, sulphur deficiency in crops has often been reported since the importance of sulphur was well recognized. After Story and Leach (1933) reported the symptom of “tea yellow” caused by deficiency in sulphur, Southern (1969) further reported deficiency symptoms of sulphur in tea plants in detail and reported the critical sulphur level in plant tissue. The sulphur study and management in tea plant is growing in importance for increased tea yield and improved tea quality. Ye (1992, 1993) and Ye et al. (1994) reported some findings on the effect of sulphur on the metabolism of carbon and nitrogen and compared the methods to measure nitrate reductase. But they only reported that sulphur application could affect yield and improve quality of green tea and individually explained the relationship between sulphur and carbon as well as the relationship between sulphur and nitrogen. They never combined them to explain how carbon and nitrogen affect the quality of green tea. The present paper attempts to ascertain the mechanism of sulphur in increasing the yield and improving the quality of green tea from the point of primary and secondary metabolism by the assay of nitrate reductase, phenylalanine ammonia-lyase and polyphenol oxidase using the results of two-year field experiments.

## 2. Materials and Methods

36-year-old green tea trees (*Camellia sinensis* (L.) O. Kuntze, variety of cloned Ju Huachun ) in the tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, China (30°16' north latitude and 120°12' east longitude) were used for this study. Field experiment about sulphur nutrition was carried out in 2002 and 2003. Soil type was the Red Soil (Ultisol, typic plinthodults). The chemical properties of the soil are shown in Table 5.1. The treatments consisted of two S fertilizer rates (0, 50 kg S ha<sup>-1</sup> a<sup>-1</sup>), and formed a 2×4 completely randomized block design with four replications. Ammonium sulphate as the source of fertilizer S was applied as basal fertilizer in each late autumn. Each treatment received a basal application of 200 kg ha<sup>-1</sup> a<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 250 kg ha<sup>-1</sup> a<sup>-1</sup> K<sub>2</sub>O and the resources were calcium magnesium phosphate and potassium chloride, respectively. Urea was applied to bring the total N supply to 600 kg N ha<sup>-1</sup> a<sup>-1</sup>. The application of N fertilizer was described in Chapter 1.

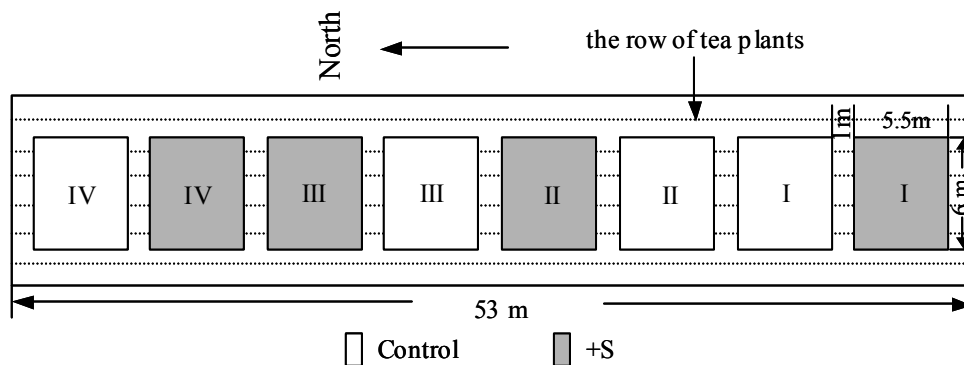


Fig. 5.1 Sketch of experimental field set-up for sulphur experiment in tea garden of Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang Province, P. R. China.

The measurement of soil chemical properties, yield constitution and quality of tea flushes see Chapter 1. The measurement of mineral nutrient content in tea flushes, ascorbic acid and dehydroascorbic acid and the assay of nitrate reductase (NR), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) activities and statistical analysis of data were described in Chapter 2. The composition of amino acids was determined with amino acid analyser (Biochrom 30) according to Reins et al. (1991).

Table 5.1. The chemical properties of the soil in the experimental site.

Soil depth (cm)	Available sulphate <sup>§</sup> (mg kg <sup>-1</sup> )	Total N (%)	Available P* (mg kg <sup>-1</sup> )	Exchangeable K <sup>‡</sup> (mg kg <sup>-1</sup> )	Exchangeable Mg <sup>‡</sup> (mg kg <sup>-1</sup> )	Organic matter (%)	pH <sub>H2O</sub>
0-20	16.55	0.05	141.00	46.58	17.00	3.68	3.80
20-40	34.54	0.06	64.20	27.78	14.39	1.87	3.67

\*: The extractant for P is 0.03 mol L<sup>-1</sup> NH<sub>4</sub>F and 0.025 mol L<sup>-1</sup> HCl;

‡: The extractant for K, Mg is 1.0 mol L<sup>-1</sup> NH<sub>4</sub>OAc;

§: The extractant for SO<sub>4</sub><sup>2-</sup> is 0.01 mol L<sup>-1</sup> Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> and 2 mol L<sup>-1</sup> HOAc.

### 3. Results

#### 3.1. Effect of sulphur on yield and quality

Application of sulphur showed the tendency to increase the fresh yield of spring and autumn, though the difference was not significant (Fig. 5.2). The vigorous nitrogen metabolism resulted in the increased production in amount. The application of sulphur slightly increased the contents of free amino acids in tea flushes, especially for those in 2002, which were increased by 5.1 % and 7.5% in both spring and autumn, respectively (Table 5.2). Although no significant effect of sulphur on the tea quality-determined constituents was detected, sulphur could still decrease TP/AA ratio (the ratio of tea polyphenols to amino acids). TP/AA ratio was decreased by 4.5% and 8.2% in the spring and autumn of 2002, respectively. In 2003, sulphur also had some effect on free amino acids and TP/AA ratio, but not as significant as those in 2002 (Table 5.2). Sulphur increased the concentrations of total ascorbate (AsA+DAsA), but a higher proportion of total ascorbate was in reduced form especially in autumn of 2003 (Fig 5.3).

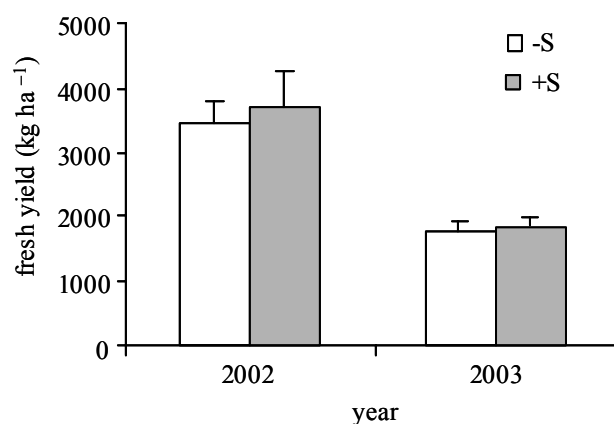


Fig. 5.2 The effect of sulphur fertilizer on the fresh yield of green tea in the spring and autumn of 2002 and 2003.



Table 5.2 The effect of sulphur on the contents of amino acids (AA, %), tea polyphenols (TP, %), the ratio of tea polyphenols to amino acids (TP/AA) in tea flushes of the spring and the autumn in 2002 and 2003.

	2002				2003			
	spring		autumn		spring		autumn	
	-S	+S	-S	+S	-S	+S	-S	+S
AA	1.77b <sup>§</sup>	1.86b	0.67a	0.72a	1.33b	1.40b	0.85a	0.87a
TP	37.23b	34.96b	28.40a	28.45a	37.63b	38.10b	35.31a	35.48a
TP/AA	19.81a	18.91a	44.02b	40.40b	28.42a	27.56a	41.51b	41.14b

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means over seasons and sulphur levels within one year are not significantly different at  $p < 0.05$  ( $n=4$ ).

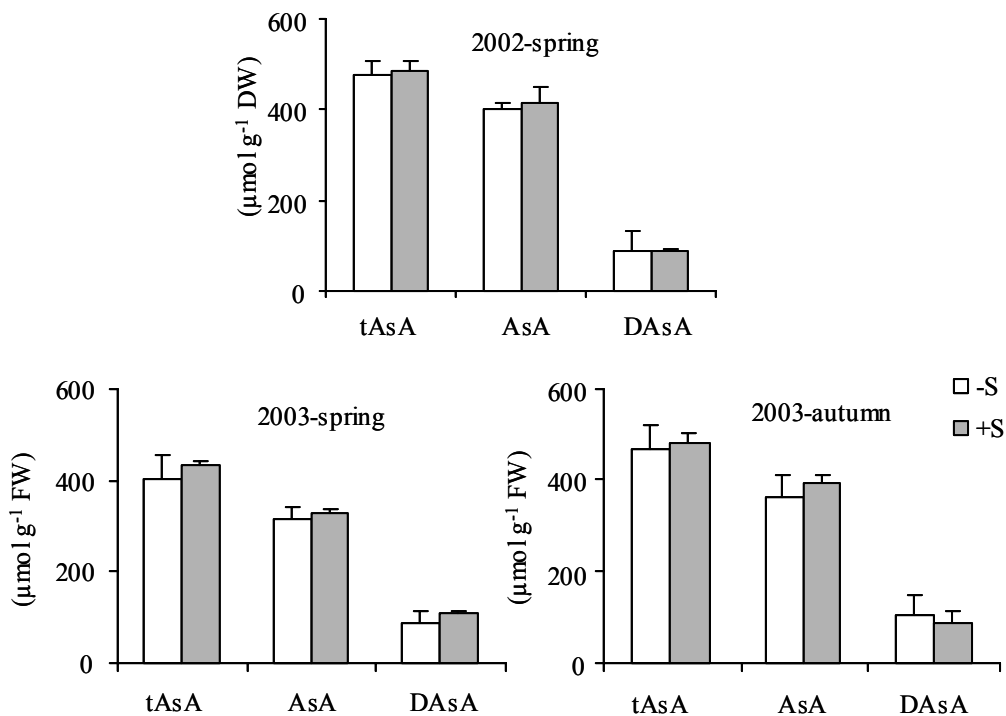


Fig. 5.3 The effect of sulphur on the total ascorbic acid (tAsA), ascorbic acid (AsA) and dehydroascorbic acid (DAsA) in the spring tea flushes (one bud and one leaf) of 2002 and in the spring and autumn tea flushes (one bud and one leaf) of 2003.

### 3.2. Effect of sulphur on mineral nutrient content in flushes

Sulphur had no significant effect on the total contents of S, N, P, K within one season, though the contents in the treatment with sulphur were slightly higher than those without sulphur (Table 5.3). However, the differences between seasons were quite significant. On average, the ratios of N:S were 19.8 and 18.3 in the spring and autumn of 2002, respectively and 16.7 and 15.6 in the spring and autumn of 2003, respectively. Total

content of P was significantly lower in autumn than that in spring in both years. On average, the ratios of P/S were 2.3 and 2.1 in the spring and autumn of 2002 and 2.2 and 1.7 in the spring and autumn of 2003, respectively.

Table 5.3. The effects of sulphur on the total contents of N, S, P and K in tea flushes in the spring and the autumn of both year.

Nutrient content (%)	2002				2003			
	spring		autumn		spring		autumn	
	-S	+S	-S	+S	-S	+S	-S	+S
N	5.53b <sup>§</sup>	5.35b	4.00a	4.03a	5.84b	5.83b	4.93a	4.89a
S	0.28b	0.27b	0.22a	0.22a	0.35b	0.35b	0.31a	0.32a
P	0.63b	0.63b	0.45a	0.46a	0.76b	0.76b	0.52a	0.52a
K	1.18a	1.20a	1.30b	1.32b	0.71b	0.71b	0.56a	0.56a

<sup>§</sup>: numbers followed by same small letters indicate that the treatment means over seasons and sulphur levels within one year are not significantly different at  $p < 0.05$  ( $n=4$ ).

### 3.3. Effect of sulphur on the activities of PPO, NR and PAL

The activity of PPO (polyphenol oxidase) was lower with sulphur supply than that without sulphur and its activity in spring was lower than that in autumn (Fig. 5.4). NR (nitrate reductase) is the key enzyme of N metabolism and PAL (phenylalanine ammonia-lyase) is the limiting enzyme of the secondary metabolism in tea plants. Generally, sulphur increased the activity of NR, because NR activity was also slightly increased in summer (Fig. 5.5 and Fig. A11). PAL activity was increased by the sulphur application (Fig. 5.5) as well.

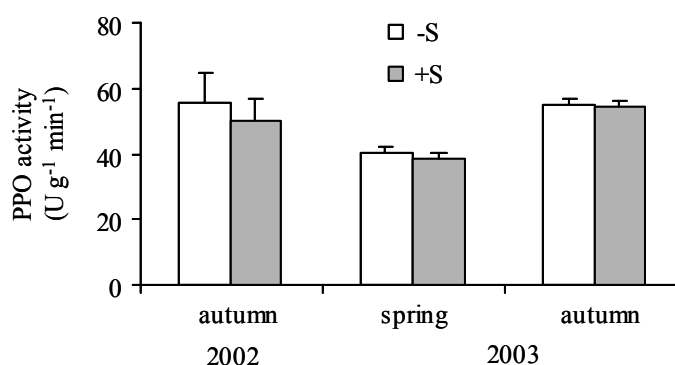


Fig. 5.4 The effect of sulphur on the activity of polyphenol oxidase (PPO) in tea flushes in the autumn of 2002 and in the spring and the autumn of 2003.

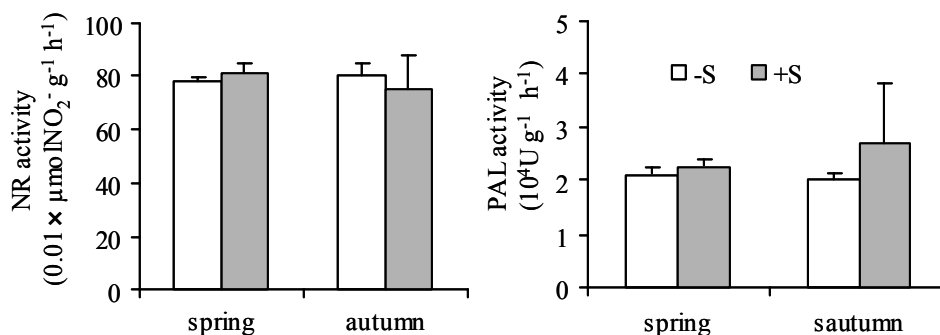


Fig. 5.5 The effect of sulphur on the activity of nitrate reductase (NR) in the 1<sup>st</sup> leaf and phenylalanine ammonia-lyase (PAL) in the tea flushes in the spring and the autumn of 2003.

### 3.4. Effect of sulphur on soil available sulphate and pH

The application of sulphur only significantly increased the available sulphate content in soil in 2003. In 2002, it only increased the available sulphate content slightly. Sulphur had no significant effect on the soil pH in both depth (Table 5.4).

Table 5.4 The available sulphate content and pH<sub>H2O</sub> in 0-20 cm and 20-40 cm soil in the late autumn of 2002 and 2003.

	Depth (cm)	2002		2003	
		-S	+S	-S	+S
Available sulphate (mg kg <sup>-1</sup> )	0-20	28.29a	33.21a	33.60a	52.75b
	20-40	31.32a	36.11a	29.23a	41.24ab
pH <sub>H2O</sub>	0-20	4.57a	4.87a	3.79a	4.06a
	20-40	4.23a	4.29a	3.79a	3.86a

§: numbers followed by same small letters indicate the treatment means over depths and sulphur levels within one year are not significantly different at  $p < 0.05$  ( $n=4$ ).

## 4. Discussion

### 4.1. Responses of yield, quality and mineral nutrient to sulphur

Sulphur is one of 16 essential elements for plant growth. When sulphur is deficient, both yield and quality suffer. In this study, sulphur application could slightly increase the fresh yield (Fig. 5.2), increase the content of amino acids, decrease the content of tea polyphenols and the TP/AA ratio (Table 5.2). But it is evident that the effect of sulphur on increasing yield and improving quality was not significant. 16 mg kg<sup>-1</sup> of soil available sulphate is normally regarded as the critical sulphur level (Liu, 1994 in Geng, 2001). But at the beginning of this experiment, the soil available sulphate content in 0-20 cm and

20-40 cm in this site was not deficient (Table 5.1). There was no significant effect of sulphur detected after the application in this site.

The functions of sulphur within the plant are related closely to those of nitrogen (N) and the two nutrients are synergistic. Sulphur content in leaf is 0.08-0.3%, the critical level is 0.12% (Ceccotti, 1996). The ratios of nitrogen, phosphorus and sulphur of the absorption by tea plant were N:S=10:1, P:S=0.6:1, respectively (Geng, 2001). Natesan and Ranganathan (1986) reported that tea could grow normally when the ratios of N to S and P to S were 9 and 1.5, respectively, which could be regarded as the standard to indicate that the tea plant was deficient in sulphur. N:S ratio of 17:1 was regarded as the critical level for protein production (Ceccotti, 1996). In this study, the N:S ratios were 15.9-19.8:1 and 15.3-19.8:1 in the absence and presence of sulphur in both years, respectively (Table 5.3). The N:S ratios were even higher than those in the study of Thomas et al. (2003), in which the N:S ratios were 14:1 and 8:1 in the absence of and presence of sulphur (Table 5.3). The P:S ratios were 1.7-2.3:1 without sulphur and 1.6-2.3:1 with sulphur. Although the P:S ratios were gradually declining, they were still much higher than the suitable ratio of 0.6:1 mentioned by Geng (2001). Normally the application of 20 kg S ha<sup>-1</sup> a<sup>-1</sup> showed a positive correlation with yield increases. However, Mkwaila thought once 20 kg S ha<sup>-1</sup> application per 3 years was enough to meet the demand of tea growth to sulphur. But in the research of Malavolta et al (1987), 30 kg S ha<sup>-1</sup> a<sup>-1</sup> was recommended for the perennial crops. The results of Ye (1993) indicated that the activity of root system and fresh yield would increase with the increased rates of sulphur applied when the sulphur applied was lower than 71.2 mg kg<sup>-1</sup>. Some other findings indicated that the fresh yield had no significant relationship with total S content, but had significantly linear correlation with the N:S ratio. This would indicate that sulphur uptake of tea plants is internally regulated such that the optimum ratio of N to S is established (Gohain and Dutta, 1994).

#### **4.2. Effect of sulphur on enzyme activities**

The compounds which play the major role in taste are tea polyphenols, amino acids and polysaccharides (Chen and Yu, 1994). PPO is one of the key enzymes for the metabolism of polyphenols, the decreased activity of PPO after the sulphur application indicated that sulphur actually lowered the rate of carbon metabolism, which resulted in the basic metabolites were transformed to the nitrogen metabolites (the relationship between PPO and TP see Chapter 2). Less tea polyphenols were beneficial to improve quality. Although PAL is often regarded as a key step in the biosynthesis of the flavonoids (Lister and

Lancaster, 1996), when carbon availability cannot be ignored and played an important role, PAL was more related to N metabolism, such as Phe and Tyr. The changes of the enzyme activities were consistent with the report that the activities of NR and PAL would increase with the increasing application of sulphur within given sulphur levels (Ye, 1993) and the increased activities of enzymes were all beneficial to increase the N metabolism and then resulted in the accumulation of amino acids, though the increase of the amino acids was not significant with sulphur supply in this study. Moreover, sulphur significantly improved the formation of asparagine and increased the content of theanine in tea flushes, especially in the spring of 2002, though sulphur had no significant effect on the other components of free amino acids (Table A1). The promotive effect of sulphur on the formation of theanine and asparagine was related to the tricarboxylic acid (TCA) cycle. Although sulphur is not directly involved in the synthetic pathways of their precursors of ethylamine from L-phenylalanine, L-glutamic acid, oxaloacetic acid and glutamic acid, oxaloacetic acid and  $\alpha$ -ketoglutarate are both supplied by the TCA cycle. However, acetyl-coenzyme A as an enzyme containing sulphur by supplying two acetyls to oxaloacetic acid to form citric acid directly involved in the TCA cycle. So deficient sulphur could influence the synthesis of acetyl-coenzyme A and then affect the synthesis of theanine and asparagine. This might also be the mechanism how sulphur affect the metabolism of nitrogen and one of the reasons why sulphur could influence the content of amino acids in tea flushes, though the sulphur effect was not significant in this study. Moreover, the response tendency of the amino acids and TP/AA in both spring and autumn to sulphur indicated that the influence of sulphur on the tea quality was not only currently effective but also residually effective.

#### **4.3. Effect of sulphur on the antioxidant protection system of green tea**

The increased vitamin C content after the application of sulphur indicated that sulphur could promote the formation of antioxidant protection system to avoid the free radical damage. Moreover, the contents of vitamin C in autumn were higher than those in spring, which was consistent with the results of Table 4.8 and Table 4.9 in Chapter 4, and further indicated the importance of vitamin C in the antioxidant protection (Laudicina and Marnett, 1990). Meanwhile, a higher proportion of the total ascorbate in the reduced form (AsA), especially in autumn of 2003 (Fig 5.3) also indicated that a higher regeneration potential of oxidized ascorbic acid (DAsA) in the sulphur deficient flushes was caused by the application of sulphur. Moreover, polyphenols are also involved in the non-enzymatical cycle of ascorbate and dehydroascorbate (Fig. 5.6). The investment of

flavonoids resulted in the increase of ascorbic acid. It also could be used to explain why sulphur application could decrease the content of tea polyphenols and increase the proportion of ascorbate.

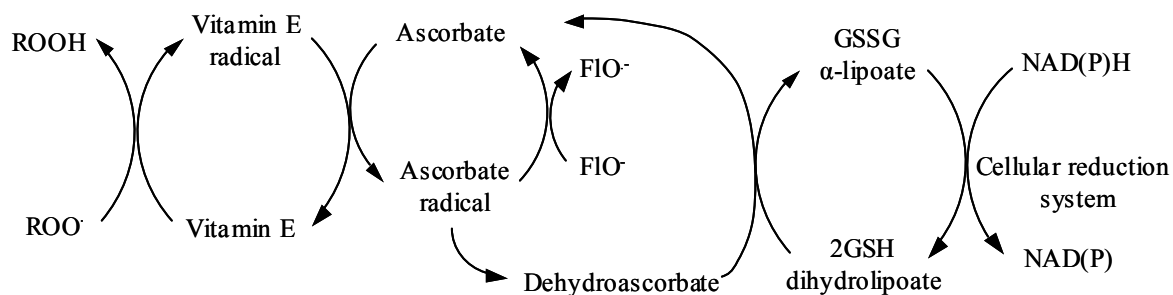


Fig. 5.6 Scheme of ascorbate and vitamin E recycling pathways. Ascorbate regenerates vitamin E from its radical form, generating the ascorbate radical. Ascorbate is regenerated from its radical by thiols, such as GSH (enzymatically) or dihyrolipoate (non-enzymatically, NAD(P)H-dependent). Proposed involvement of flavonoids (FIO·) in ascorbate regeneration from ascorbate radical, resulting in flavonoid radical (FIO·) formation.

#### 4.4. Effect of sulphur on soil available sulphate and pH

Sulphur of different forms all could improve the available sulphate content in soil. When  $20 \text{ kg S ha}^{-1} \text{ a}^{-1}$  was applied, the available sulphate in soil in 6 years could be increased by  $4 \text{ mg kg}^{-1} \text{ a}^{-1}$  on average (Geng, 2001). Because of sulphur fertilizer is easily to be lost from the soil and over supply of sulphur not only is a waste but also can result in the acidification of soil. In this study, at the beginning of the experiment, soil available sulphate content in 0-20 cm soil was just at the threshold level for the sulphur deficiency and in the deeper layer, soil available sulphate (Table 5.1) was sufficient according to the critical soil available sulphate level  $16 \text{ mg kg}^{-1}$  (Liu, 1994 in Geng, 2001). After the application of sulphur fertilizer, in the first experimental year, some improving effect from sulphur was detected, but in 2003, the positive effect sulphur was reduced. Because at the end of the first experimental year, the content of sulphate in soil was sufficient (Table 5.4), sulphur application had less effect on the yield and the quality in 2003 (Fig. 5.2 and Table 5.2). However, at the same time of sulphur content was increased, soil pH was also slightly reduced by sulphur application in 2003 compared to 2002 (Table 5.4). The acidification of sulphur returned to affect the normal growth and metabolism of the tea plants. This is why a smaller sulphur effect on the tea quality was detected in 2003 than that in 2002 (Table 5.2).

In this study, two conclusions can be made. One is that the sulphur was not so deficient in this research site and the other is that high sulphur applied rate was harmful to the growth of tea plants for its changing the N:S ratio. Only when the optimum N:S ratio was obtained, sulphur can have its effect.





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## General discussion

### **The effect of interaction between N supply and light intensity on yield and quality**

Generally, N application rates had no significant effect on yield and quality (Fig. 1.2 and Table 1.4). The results in Table 2.1-Table 2.3 indicated that shading decreased the yield and improved the quality, that is, shading increased the content of amino acids and decreased the TP/AA ratio, which showed significantly negative linear regression with green tea quality (Shi and Liu, 1987). Our results are consistent with that of Othieno (1983). In 2003, the effect of shading on improving quality was not as significant as that in 2002, which may result from the high transmitted light intensity inside the shading net after shading in extreme high irradiance in 2003 (Table 1.1 and Table 1.2).

The results of mineral nutrient content in tea flushes indicated that shading had no significant effect on total content of N, but shading could increase the total contents of P, K in tea flushes (Table 2.6 and Table 2.7) and Mg, Ca, Al and Mn (data not shown), which were consistent with the results of Zhao and Oosterhuis (2000). The study of Duan and Guo (1992) indicated that in tea gardens shading decreased air temperature by 0.5-4.0°C in summer. The absolute content of soil water and relative humidity of the air were increased 17-40% and 2-20%, respectively, because of the lower velocity and less evaporation after shading. Moreover, low light intensity can keep tea plants growing in a low speed, which indirectly made the tea flushes to be kept tender for a longer time. So the changes of microclimate under shading were the main reason causing the increased uptake of most nutrients.

The relationship between NR, PAL, PPO activities and light intensity as well as the metabolism of amino acids and polyphenols were described in Fig. 2.6. NR activity in the 1<sup>st</sup> leaf was much higher than in 3<sup>rd</sup> leaf (Fig 2.2), which is consistent with the result that nitrate reductase showed the highest activity in the 1<sup>st</sup> leaf (Wu and Wu, 1993). There was no obvious correlation between NR activity and N application rate in soil, though the NR activity can be induced by the substrate of nitrate and nitrate nitrogen in soil showed a strongly linear relationship with N application rate (Fig. A9). However, there was a significant effect of light intensity on it. Low light intensity resulted in relative lower NR activity in leaves of both positions, because light is an important regulatory signal for NR and NiR expression (Crawford, 1995). Decreased NR activity could partly hinder the synthesis of some aromatic amino acids, in which the precursors of cinnamic acid, such as Phe and Tyr were included.

Light has an inducible effect on PAL activity and synthesis or linkage of A ring and hydroxylation of the B ring are promoted by light (Forrest, 1969). The decreased activity of PAL caused by shading verified this finding (Fig. 2.3). As was shown in Fig. 2.6, i) The *N*-ethyl carbon of theanine is specially incorporated into the phloroglucinol nucleus of catechins in tea shoots. This incorporation is greatly controlled by light (Kito et al., 1968). These results may well explain the relationship between contents of theanine and catechin in shaded and exposed tea leaves. ii) Light may be needed for the degradation of theanine in the restricted site of theanine degradation, where the *N*-ethyl carbon of theanine may be converted to the phloroglucinol nucleus of catechins probably by the condensation mechanism via acetyl-CoA (Kito et al., 1968). iii) Less *N*-ethyl carbon is required by the synthesis of catechin caused by low activity of carbon metabolism at low light intensity, which resulted in the accumulation of theanine. v) The growth of tea plants might be inhibited by shading and less theanine is consumed to its further metabolism. So more free theanine is left. However, according to the results in this study, no definite explanation can be made. So it is still necessary to do much more work on this aspect to support any above hypothetical explanation.

Plant PPO is another important enzyme related to polyphenols. Ye et al. (1994) reported some information about PPO. But in his study, it was simply thought that the decreased PPO activity was not beneficial to the metabolism of carbon, which inhibited synthesis of polyphenols. This study also indicated the shading could significantly increase the activity of PPO in tea flushes (Fig. 2.3). While the function of PPO in the chloroplast remains unresolved, the finding of PPO in PS II particles increased the need to assess any role that PPO may play in the electron transport of that photosystem, whether structural or functional (Hind et al., 1995). Thus, the increased PPO activity in shaded growth condition could not be merely and simply linked to the decrease of tea polyphenols in tea flushes, its role in biological oxidation within tea plants also should be taken into consideration, because it is thought to function at the step from respiratory pigment oxidized to O<sub>2</sub> in situ. As for the physiological function of PPO in green tea, it still needs much further work on it. In any case, the combination of these three enzymes could provide some threads to explain why shading can improve the quality of tea, though the explanations are not overall.

The effect of interaction between light intensity and N supply on gas exchange and antioxidant protection system

Plants are capable of adaptation to their light environmental conditions (Berry, 1975). Under shading, leaves became thinner and larger, resulting in a higher specific leaf area (Tables 2.8-2.10). This modification to thinner and larger leaves is a common adaptation to low irradiance (Higuchi et al., 2001) and might be looked at as a strategy to maximize light interception, that is, to maximize the net rate of energy capture (Givnish, 1988). Leaf chlorophyll contents on the basis of unit leaf area generally indicated a tendency to increase under shade. The increase of chlorophyll content and the decrease of chlorophyll a/b (Table 2.8) were consistent with the other reports (Anderson, 1986; Chartzoulakis et al., 1993 and Schaffer and Gaye, 1989). This increasing tendency of chlorophyll content might also be an acclimation to low light environment to enhance the light use efficiency. The increase in chlorophyll concentration, especially chlorophyll b of shaded leaves might be associated with the adaptation of leaves to low light environment (Barker, 1985 in Zhao and Oosterhuis, 1998) to intercept more light energy, or with a decreased chlorophyll degradation rate (Hale and Orcutt, 1987 in Zhao and Oosterhuis, 1998). Increasing the chlorophyll b content may also increase the proportion of blue light captured by photosystem II (Anderson, 1982). It has also been suggested that shadelight is more effective in exciting photosystem I than sunlight and that, to redress this imbalance, shade plants have a larger ratio of photosystem II to photosystem I, in terms of both chlorophyll or reaction centers (Björkman, 1981 in Evans, 1988). Meanwhile light-harvesting chlorophyll a/b protein complex (LHC) that captures and transmits the light energy in photosystem locates in photosystem II. The plant grown in natural shade has more LHC and the proportion of chlorophyll associated with LHC was increased. So the content of chlorophyll b was increased with the relative increasing of LHC. Moreover, the relative increase in chlorophyll b may enhance the ability of shaded leaves to capture and utilize the light containing wavelengths that are enriched in the infra-red region of the spectrum (Chartzoulakis et al., 1993).

High light intensity decreased stomatal conductance ( $g_s$ ), intercellular  $CO_2$  concentration ( $C_i$ ) and transpiration rate (E) again (Table 2.10), which is consistent with the reported results of fruit trees (Higgins et al., 1992). Shading was effective to decrease the leaf temperature, easing of water stress. The effect of shading on increasing  $g_s$  may be attributed to increase net photosynthesis (Pn), compensating for the effect of low light intensity (Higuchi et al., 2001). In this study, only the Pn with the application of 600 kg N ha<sup>-1</sup> a<sup>-1</sup> within the range of 100-1500 PPFD followed the rule, in control the results were completely did not (Fig 2.4). Moreover, light-saturated rate of net  $CO_2$  assimilation at

ambient  $P_a$  ( $A_{max}$ ) and light compensation points ( $I_c$ ) in both N levels were all lower in low light intensity than those in full sun (Table 2.10). So the fact that  $P_n$  including the  $A_{max}$  and  $I_c$  as well as dark respiration rate ( $R_d$ ) and leaf nitrogen content per unit leaf area were all lower in shaded leaves (Fig. 2.4 and Table 2.10) suggested that either the light reaction in shaded leaves was less efficient than that in exposed leaves, though generally the chlorophyll content was increased and leaf morphology or chloroplast structure were more suitable to capture light energy. This is one of the reasons why shading reduced the yield. Combination enzyme activities with photosynthesis, we can initially explain why shading can reduce the yield at the same time of improving the quality.

Vitamin C (ascorbic acid and dehydroascorbic acid) is an indicator of fresh and high quality green tea (Liang et al., 1996) and a major antioxidant in biological systems. It has been reported that vitamin C can synergistically regenerate vitamin E, resulting in an enhancement of antioxidant activity in membranes, thereby preventing them more efficiently from being oxidized under free radical oxidative stress (Cossins et al., 1998) (Fig. 5.6). The ratios of ascorbic acid to dehydroascorbic acid (AsA/DAsA) in full sun were 107% and 7% lower than those under shading in 2002 and 2003, respectively (Table 2.4 and Table 2.5). The higher AsA/DAsA ratio under shading in both years implied that low light intensity could have reduced the occurrence of free radical-associated damage and that high-content ascorbic acid to inhibit the free radical-associated damage was not as necessary as in full sun. Moreover, the higher content of vitamin C in 2003 than that in 2002 indicated the effect of light on the formation of vitamin C and stressed the function of vitamin C as an antioxidant.

It is well documented that light energy harnessed by chlorophyll, which cannot be dissipated via photosynthesis on time, can lead to the formation of oxygen free radicals, especially under water-limiting conditions (Kaiser, 1987; Larson, 1988; Navari-Izzo, et al., 1997). The investigation into the role of light during desiccation (Farrant et al., 2003) suggests that APX and GR activity increased only in plants water-stressed in the light. Plants water-stressed in the dark had no measurable activity. The lack of increased antioxidant activity in plants could have allowed for accrual of free radical-associated damage, which in turn contributed to their death. Our study also showed the same results in which the activities of APX, DHAR and GR activity in full sun under the high irradiance and drought conditions all were all slightly higher than those under shading (Fig 2.5), which implied that for the shaded leaves, a light driven regulation of the antioxidant and anthocyanin protection system may not be as vital as the exposed leaves. So it can be

at least partly used to explain why the contents of ascorbic acid in tea flushes (Table 2.4, Table 2.5) and in mature leaves (Table 2.11) under shading were lower than that in full sun. It also indicated that shading could reduce the risk of free radical damage for tea plants.

### **Diagnosis of N status and the effect of shading on the N diagnosis**

Yield, quality and economic profit are the three most important aspects to green tea production. Large doses of nitrogen fertilizers are usually applied in tea cultivation, because the quality of green tea is markedly correlated with the content of total nitrogen or total free amino acids (Watanabe, 1995). The price of made tea is also highly correlated with the quality, so yield and quality decide the final profit of the tea growers. In Chapter 3, according to the investigation in 500 sites in Zhejiang province from 1996 to 2000, the price of tea ranged from 9 RMB kg<sup>-1</sup> to 167 RMB kg<sup>-1</sup>. The profit of tea growers is strongly fluctuant from minus to 9100 RMB ha<sup>-1</sup> according to the calculation in Table 3.1. So in the study of diagnosis of N status, 95% of the maximum yield (95%Y<sub>max</sub>) was considering as the optimum yield in order to get the high profit. 810 kg N ha<sup>-1</sup> a<sup>-1</sup> was calculated from 95% Y<sub>max</sub> as the optimum N application rate for high quality green tea. The whole model of N diagnosis was established based on the results of spring, summer and autumn. Although soil N<sub>min</sub> could significantly index total fresh yield and quality (Fig. 3.7 and Fig. 3.8), plant analysis was regarded a faster and more sensitive method (Lacroix and Cousins, 1997), which was verified by our results, especially in summer. The results in Fig. 3.7 suggested that soil N<sub>min</sub> could not sensitively reflect the minor difference of yield and quality in shaded and exposed conditions, but plant analysis reached it (Table 3.12). In the very early spring, the diagnosis was not so necessary as those in spring, summer and autumn, though the NO<sub>3</sub><sup>-</sup> in top old leaves (the leaves were on the brown stems and grew up from the former year) was regarded as a good indicator (Fig. 3.11 E, Fig. 3.13 E and Fig. 3.18 D). 30% N of the treatment was applied before spring tea, which was sufficient to the growth of tea plants in the early spring. Konishi et al. (1978) and Watanabe (1988) observed that the nitrogen absorbed in autumn affected the growth of new shoots and the nitrogen absorbed in spring affected the increase of total nitrogen and total amino acid contents in new flushes. So in early spring, tea plants could use the basal nitrogen fertilizer applied in the late former autumn. With the time going on, NO<sub>3</sub><sup>-</sup> and AAN (amino acid nitrogen) in young stem and new fully expanded leaves in the plucking

period were good indicators in spring. They could not only reflect the level of soil  $N_{\min}$  (Fig. 3.18) and index total yield (Fig. 3.11), but also could index the quality (Fig. 3.13). In autumn, only  $NO_3^-$  and AAN in mature leaves were suitable indicators because AAN in young stem could not reflect the soil  $N_{\min}$  level (Fig. 3.20 B) and  $NO_3^-$  in young stem could not index the quality any more (Fig. 3.14 A). In summer, although  $NO_3^-$  and AAN in young stem and mature leaves in shaded and exposed conditions were all significantly correlated with soil  $N_{\min}$  (Fig. 3.22), the AAN in young stem (Fig. 3.15 D) and  $NO_3^-$  and AAN in mature leaves (Fig. 3.10 F, H) in full sun and AAN in mature leaves under shading (Fig. 3.15 G) could not index the total yield. In addition, AAN in young stems (Fig. 3.16 D) and  $NO_3^-$  and AAN in mature leaves (Fig. 3.16 F, H) in full sun could not index the quality. Consequently  $NO_3^-$  in young stem was the most suitable indicator in both shaded and exposed conditions in summer. Generally, shading only had significant effect on the optimum N application rate (810 kg N ha<sup>-1</sup> a<sup>-1</sup> in full sun and 700 kg N ha<sup>-1</sup> a<sup>-1</sup> under shading) (Fig. 3.2) and the critical level of indicators (Table 3.2), but had no effect on critical soil  $N_{\min}$  and optimum N application rate for summer tea (Table 3.3). In three seasons, the soil  $N_{\min}$  was significantly linearly correlated with N application rate within the season (Fig. 3.17, Fig. 3.19 and Fig. 3.21).

Table G1 The critical contents of the indicators (mg L<sup>-1</sup>) and soil  $N_{\min}$  (kg N ha<sup>-1</sup>) in spring, summer and autumn of 2003 and the optimum N application rate within the season ( $N_{\text{opts}}$ , kg N ha<sup>-1</sup>). (AAN : amino acid nitrogen; YS: young stems; NFEL: new fully expanded leaves; TOL: top old leaves, which were on brown stems and grew up from the former year; ML: mature leaves).

Season	Light	Indicator	Content	Soil $N_{\min}$	$N_{\text{opts}}$
Spring		$NO_3^-$ in YS	30-33		
		$NO_3^-$ in NFEL	6-7	200-260	240-340
		$NO_3^-$ in TOL	10-11		
		AAN in NFEL	714-732		
Summer	S	$NO_3^-$ in YS	32-51	180-230	140-240
	E	$NO_3^-$ in YS	20-33		
Autumn		$NO_3^-$ in ML	5-6	320-430	130-230
		$NO_3^-$ in ML	495-510		

S: shaded condition; E: exposed condition

According to this study, all the results related to nutrient diagnosis were shown in Table G1. If based on the optimum N application rate of 810 kg N ha<sup>-1</sup> a<sup>-1</sup>, the partition of N rate

in spring (30%) and autumn (20%) were 240 kg N ha<sup>-1</sup>, 160 kg N ha<sup>-1</sup>, respectively. Because of the strong effect of shading on the optimum N application rate in summer, 810 kg N ha<sup>-1</sup> a<sup>-1</sup> in full sun and 700 kg N ha<sup>-1</sup> a<sup>-1</sup> under shading, the 20% of them were 160 kg N ha<sup>-1</sup> in full sun and 140 kg N ha<sup>-1</sup> under shading, respectively. So the proportion of N application rate in three seasons should be adapted correspondingly according to this study. For spring tea, the N application rate was only in the lower limiting threshold of  $N_{\text{opts}}$ , but for autumn tea, the N application rate in the range of  $N_{\text{opts}}$ . However, in spring, the intensive plucking can remove much more nitrogen from the production system than that in autumn; in summer, if the shading measure is taken and the summer tea is plucked to make green tea, the applied N is not sufficient, because the N application rate was only in the lower limiting threshold of  $N_{\text{opts}}$  in summer. Therefore, according to this study, the application rate for autumn tea can be reduced and should be applied for spring and summer tea, especially to spring tea. So far, the diagnosis system for tea is hardly developed and this study is not the ultimate solution, but this study supplied a very important step to establish the diagnosis system in tea plants. Much more work in this aspect is still needed.

If the highest total content of N of 6.5%, 4.8% and 4.6% in tea flushes in spring, summer and autumn, respectively and the highest total content of N of 3.5% in pruned tea were considered to calculate N removal, N removal was 160-200 kg N ha<sup>-1</sup> a<sup>-1</sup> from control to the highest N level treatment by plucking and pruning within one year. The loss of N from tea production system in this study was almost twice as much as that in black tea production system (90-120 kg N ha<sup>-1</sup> a<sup>-1</sup>) (Wanyoko, 1981 in Owuor et al., 1987). This difference was mainly caused by the lower N content (3.0-3.4%) in tea flushes in his study. Another possible reason was that a large amount of N was taken away by the pruned tea flushes in this study. But the pruned tea flushes were returned to the production system as the organic matter after pruning. Therefore, the real N removal was much less than that calculated. Without considering the pruning removal, only 40-80 kg N ha<sup>-1</sup> a<sup>-1</sup> was removed by plucking (Fig. 3.3). To compare this with 90-120 kg N ha<sup>-1</sup> a<sup>-1</sup> in black tea, it is evident that the N removal in green tea was much less than black tea, which results from the different plucking standards between green tea (one bud and one or two leaves) and black tea (one bud and three or four leaves). Othiento (in Owuor, 1987) estimated that the efficiency of nitrogen uptake by the tea plants was less than 50% under Kenya tea growing conditions. Considering the soil  $N_{\text{min}}$  (50-540 kg N ha<sup>-1</sup> a<sup>-1</sup>) and calculation of N removal by plucking (40-80 kg N ha<sup>-1</sup> a<sup>-1</sup>) in this study, we can make the conclusion that large

amounts of the applied N fertilizer was lost from tea production system, especially in the high N level treatment (900 and 1600 kg N ha<sup>-1</sup> a<sup>-1</sup>). It was maybe attributed to the strong leaching due to the normally high rainfall, especially in spring and the early summer in the South of China. Moreover, denitrification also can reinforce the N loss, because it was considered to be the main process to produce N<sub>2</sub>O in acid tea soil with pH value below about 3.60 (Tokuda and Hayatsu, 2000).

### **Effect of K and Mg on yield and quality**

The empirical threshold for soil exchangeable K and Mg are 80 mg kg<sup>-1</sup> (He and He, 1993) and 40 mg kg<sup>-1</sup> (Tea Research Institute, China) in China, respectively. It is evident that the exchangeable K and Mg contents in soil were not sufficient at the beginning of this experiment in both sites (Table 4.1 and Table 4.2), suggesting that a positive effect of K and Mg on yield and quality of green tea. In Longjing43 sites, the yield could be significantly increased by the application of N, P, K and Mg fertilizers, but there was no significant difference between the treatments of N, NP, NPK and NPKMg. In summer and autumn of 2003, yield showed a strong increasing tendency with the application of K fertilizer, which implied that K functioned in resisting drought (the meteorological data in 2003 are shown in Table 1.2). In Biyun site, the yield showed the increasing tendency from control to NPKMg (Table 4.3 and Table 4.4), but differences between treatments were not significant.

In both sites, the application of N, NP, NPK and NPKMg fertilizers could slightly increase the contents of amino acids and decrease the ratio of TP/AA (Table 4.5 and Table 4.7). There was a potential that all the contents of individual amino acid were highest in the treatment receiving NPK (Table 4.6). Only the SPAD values with NPKMg (Table 4.11 and Table 4.9) were highest among the treatments, which was associated with the nutritional functions of Mg in the synthesis of chlorophyll (Marschner, 1995). But no the relationship between SAPD and yield or quality was observed in this study. In the other case, the antagonism of K and Mg caused the effect of K on quality to be inhibited by Mg. The contents of total ascorbic acid, ascorbic acid and dehydroascorbic acid in the tea flushes of Longjing43 and Biyun were not significantly affected by the fertilizers but by seasons. The average AsA/DAsA ratio of Longjing43 was 6.5, 3.7 and 5.6 in spring, summer and autumn, respectively. The NPK application could increase AsA/DAsA ratio of Longjing43 from 2.9 (control) to 4.4 on average in summer (Table 4.8). But the average



AsA/DAsA ratios of Biyun were 3.6, 4.6 and 2.2 on average in spring, summer and autumn, respectively. The NPK application could increase AsA/DAsA ratio of Biyun from 2.1 (control) to 3.8 on average in autumn (Table 4.9). So K application increased the content of reduced form of vitamin C compared to other treatments. The highest vitamin C content of Longjing43 appeared in autumn and then in summer, the lowest content was in spring; while the vitamin C content of Biyun followed the sequence from high to low: summer, autumn and spring.

At the end of 2003, in Longjing43 site, soil exchangeable Mg content was still deficient (Table 4.13), but in Biyun site, both exchangeable K and Mg contents were sufficient according to that critical standards (Table 4.14). Compared to 2002, soil available P, exchangeable K, Mg and Ca were significantly increased in 2003, even in control, on average, the soil available P, exchangeable K, Mg and Ca were averagely increased by 106%, 42%, 88% and 39% and pH was decreased by 3.7% in both sites in 2003, respectively. This strange change of soil mineral nutrient contents in both years made it difficult to explain the results obtained in this study. A large amount of sulphur powder applied is one of the reasons to cause the decreased pH, therefore resulted in the big change of soil exchangeable mineral elemental contents. Maybe this change was caused by the extreme weather in 2003. Anyway, according to this two-year study, we could not present a good explanation to it.

### **Effect of S on yield and quality**

Soil available sulphate of 16 mg kg<sup>-1</sup> is usually regarded as the critical sulphur level (Liu, 1994 in Geng, 2001). At the beginning of this experiment, soil available sulphur content was not deficient (Table 5.1). So the effect of sulphur on increasing the yield (Fig. 5.2) and improving the quality was not significant (Table 5.2). S application could increase the proportion of ascorbate in reduced form, especially in autumn of 2003 (Fig. 5.3, the relationship between ascorbate and dehydroascorbate was shown in Fig. 5.6), but the effect was still not significant. NR, PAL and PPO were assayed in this study. The same efforts were made to use them to explain the metabolism of sulphur on the yield and quality (Fig. 5.4 and Fig. 5.5). But there was no significant difference between in the absence and the presence of sulphur.

The functions of sulphur within the plant are related closely to those of nitrogen and the two nutrients are synergistic. The ratio of nitrogen and sulphur of the absorption by tea

plant is N:S=10:1 (Geng, 2001). Natesan and Ranganathan (1986) reported that tea could grow normally when the ratios of N to S was 9, which could be regarded as the standard to indicate if the tea plant is deficient in sulphur. N:S ratio of 17:1 was regarded as the critical level for protein production (Ceccotti, 1996). In this study, the N:S ratios were 15.9-19.8:1 and 15.3-19.8:1 in the absence and the presence of sulphur in both years, respectively (Table 5.3). The N:S ratios were even higher than those in the study of Thomas et al. (2003), in which the ratios of N:S were 14:1 and 8:1 in the absence of and presence of sulphur (Table 5.3). But N:S ratio in this study could not affect the protein production. Sulphur content in leaf is 0.08-0.3%, the critical level is 0.12% (Ceccotti, 1996). In this study, total sulphur content in tea flushes was high enough (Table 5.3). The results of soil available S at the end of each year also indicated that soil sulphur was sufficient after the first experimental year (Table 5.4). So it could explain why only smaller significant effect of sulphur on yield and quality was detected. Now that soil available sulphate and total content of S in tea flushes were both sufficient, why did the N:S ratio still not reach the critical ratio? It could not be explained by this study, maybe just the unbalanced uptake or unsuitable supply of N and S resulted in the higher N:S ratio, therefore affected the function of sulphur further.

### **Prospect**

The physiological functions of PPO in the metabolism of polyphenols, photosynthesis (PSII) and respiration should be given greater consideration in green tea in future studies. Although the model of the diagnosis of N status had been initially established in this study, only the  $N_{\text{opty}}$  and  $N_{\text{opts}}$  could be presented. In this study, only some basic parameters about the diagnosis in tea plants were obtained, more work is needed over a wider range of sites and years to establish a model including the establishment of the relationships between instant soil  $N_{\text{min}}$  and indicators; the consideration of economic factors to fully evaluate the response of tea plants to N fertilization; the relationship between different light intensity and diagnosis standards of the indicators under shading; the development of a new methodology to identify the critical indicators of N with higher precision, which can be used to predict the N status for growing fields. More consideration should be given to the management to increase N use efficiency by increasing the  $\text{NH}_4^+$  retention in the soil, and future studies should evaluate the suitability of nitrification inhibitors and identify the proper N application time. It is needed that a wider range of years to study the metabolism

of K and Mg to increase the yield and improve the quality, especially the synergistic effect of K and N on the metabolism of amino acids within tea plant.



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## Summary

In cooperation with the Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China (30°16' N and 120°12' E), field experiments were conducted over two years (2002 and 2003) to study the background level of nutrients in tea-grown soils and the responses of tea yield and quality to fertilization. A diagnosis system was developed to estimate plant N fertilizer demand, to optimise N management in tea gardens in spring and autumn and to study the effect of shading on the diagnosis of N status in summer tea. The impact of the application of N, K, Mg and S on yield and quality was investigated, too. To understand the direct effect of shading on tea quality, the interaction between light intensity and N supply was also studied. To extend the interpretation of the results, the gas exchange was measured.

The direct positive effect of shading on the improvement of tea quality was detected, though the yield decreased under shading. Tea flushes in lower light intensity had higher content of free amino acids, lower content of tea polyphenols, which resulted in a lower TP/AA ratio than those in full sun, especially in summer of 2002. Moreover, the tea flushes under shading had the decreased contents of total ascorbic acid, ascorbic acid and dehydroascorbic acid. Shading increased the total contents of P and K in tea flushes. The reduced activities of nitrate reductase (NR) and phenylalanine ammonia-lyase (PAL) under shading inhibited the synthesis of the precursors of cinnamic acid and catechin. Moreover, the increased polyphenol oxidase (PPO) in shaded condition might enhance the degradation of polyphenols. The combination of them can partially explain why shading could decrease TP/AA to improve the quality of green tea. Under shading, the increased leaf water content, specific leaf area and the decreased the ratio of chlorophyll a to chlorophyll b, the leaf total nitrogen content per unit leaf area were identified as typical adaptations of tea plants to low irradiance in order to capture more light energy per unit biomass. Decreased light saturated rate, light compensation point and dark respiration rate under shading explained partially why shading could decrease the yield. The decreased activity of ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase and the decreased content of vitamin C in shading implied that the light driven regulation of the antioxidant and anthocyanin protection system may not be as vital as that in full sun.

Initially, the suitability of different rapid test methods of SPAD meter, nitrate electrode and nitrate reflectrometer was compared. Nitrate reflectrometer (NO<sub>3</sub><sup>-</sup> test strips) proved most suitable to measure the NO<sub>3</sub><sup>-</sup> contents in the sap of tea tissues. Ninhydrin

colorimetric method was used to measure amino acid nitrogen (AAN, based on the standard of glutamic acid) contents in the pressed tea sap. 95% of the maximum yield was regarded as the optimum yield considering the economic profit. The optimum N application rate ( $N_{\text{opty}}$ ) of  $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  was calculated from 95% of the maximum, because it was closer to the N level at the lowest TP/AA (ratio of tea polyphenols to amino acids, which is significantly negative linear regression with quality of green tea). In spring, the critical  $\text{NO}_3^-$  contents in different organs were  $30\text{-}33 \text{ mg L}^{-1}$  in the young stem,  $6\text{-}7 \text{ mg L}^{-1}$  in new fully expanded leaves (NFEL),  $10\text{-}11 \text{ mg L}^{-1}$  in the top old leaves (leaves were on the brown stems and grew up from the former year), respectively. The critical AAN content was  $714\text{-}732 \text{ mg L}^{-1}$  in NFEL. In autumn, the critical contents of indicators were  $5\text{-}6 \text{ mg L}^{-1} \text{ NO}_3^-$  and  $495\text{-}510 \text{ mg L}^{-1} \text{ AAN}$  in mature leaves (ML), respectively. The critical soil  $N_{\text{min}}$  in 0-60 cm soil depth were  $200\text{-}260 \text{ kg N ha}^{-1}$  and  $320\text{-}430 \text{ kg N ha}^{-1}$  in spring and autumn, respectively. The optimum N application rates within a season ( $N_{\text{opts}}$ ) were  $240\text{-}340 \text{ kg N ha}^{-1}$  and  $130\text{-}240 \text{ kg N ha}^{-1}$  in spring and in autumn, respectively. In summer, shading had strong effects on the  $N_{\text{opty}}$  ( $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  in full sun and  $700 \text{ kg N ha}^{-1} \text{ a}^{-1}$  under shading) and the critical  $\text{NO}_3^-$  levels in young stem ( $20\text{-}33 \text{ mg L}^{-1}$  in full sun and  $32\text{-}51 \text{ mg L}^{-1}$  under shading). But shading had no effect on the critical soil  $N_{\text{min}}$  ( $180\text{-}230 \text{ kg N ha}^{-1}$  in both shaded and exposed conditions) and  $N_{\text{opts}}$  ( $140\text{-}240 \text{ kg N ha}^{-1}$  in both shaded and exposed conditions). If based on the optimum N application rate of  $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$ , the partition of N application rate in spring (30%) and autumn (20%) were  $240 \text{ kg N ha}^{-1}$ ,  $160 \text{ kg N ha}^{-1}$ , respectively. Due to the strong effect of shading on  $N_{\text{opty}}$  ( $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  in full sun and  $700 \text{ kg N ha}^{-1} \text{ a}^{-1}$  under shading), the 20% of them were  $160 \text{ kg N ha}^{-1}$  in full sun and  $140 \text{ kg N ha}^{-1}$  under shading, respectively. So the proportion of N application rate in three seasons should be adapted correspondingly according to this study. For spring tea, the N application rate was only in the lower limiting threshold of  $N_{\text{opts}}$  ( $240\text{-}340 \text{ kg N ha}^{-1}$ ), but for autumn tea, the N application rate was in the range of  $N_{\text{opts}}$  ( $130\text{-}240 \text{ kg N ha}^{-1}$ ). In summer, if the shading measure is taken and the summer tea is plucked to make green tea, the application N is not sufficient, because the N applied rate was only in the lower limiting threshold ( $N_{\text{opts}}$ ,  $140\text{-}240 \text{ kg N ha}^{-1}$ ) in summer. Therefore, according to this study, the application rate for autumn tea can be reduced and should be applied to spring and summer tea, especially to spring tea.

The application of potassium and magnesium fertilizers could increase the yield and improve the quality, especially for variety Longjing43 in 2003. Due to the antagonism of K and Mg, the effect of K on increasing the content of amino acids and the ratio of

ascorbic acid to dehydroascorbic acid was better than that with K and Mg together. Meanwhile, the increased of exchangeable Ca and the antagonism of Ca, K and Mg caused the limited effect of K and Mg on increasing yield and improving quality.

Sulphur application could slightly increase the fresh yield, the content of free amino acids, total ascorbate and decrease the content of polyphenols and TP/AA ratio. Meanwhile, the application of sulphur could slightly decrease the ratio of N:S, increase the activities of NR and PAL, but inhibit the activity of PPO. But relatively sufficient sulphur content in the experimental site inhibited the effect of sulphur applied on yield and quality.





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## Zusammenfassung

In Zusammenarbeit mit dem Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang province, P. R. China (30°16' N and 120°12' O), wurden Feldversuche über zwei Jahre (2002 und 2003) durchgeführt, um das Versorgungsniveau an Nährstoffen in Teeböden und die Auswirkungen von Düngungsmaßnahmen auf Teeertrag- und -qualität zu untersuchen. Es wurde ein Diagnosesystem entwickelt, um den N-Düngerbedarf der Pflanze abzuschätzen, das N-Management in den Teeplantagen im Frühling und im Herbst zu optimieren und den Effekt einer Beschattung auf die Diagnose des N-Status des Tees im Sommer zu untersuchen. Weiterhin wurde der Einfluss einer Applikation von N, K, Mg und S auf Ertrag und Qualität untersucht. Um den direkten Einfluss einer Beschattung auf die Teequalität zu verstehen, wurde ebenfalls die Interaktion von Lichtintensität und N-Versorgung studiert. Gaswechsellmessungen ermöglichten eine vertiefte Interpretation der Ergebnisse.

Der direkte positive Effekt einer Beschattung auf die Verbesserung der Teequalität konnte bestätigt werden, obwohl der Ertrag unter Beschattung abnahm. Die bei niedrigerer Lichtintensität geernteten Teeblätter besaßen höhere Gehalte an freien Aminosäuren, niedrigere Gehalte an Polyphenolen, und damit ein niedrigeres TP/AA-Verhältnis als bei denen in voller Sonne, besonders im Sommer 2002. Weiterhin wiesen die junge Teezweige unter Beschattung niedrigere Gehalte an Gesamt-Ascorbinsäure (tAsA), Ascorbinsäure (AsA) und Dehydroascorbinsäure (DAsA) auf. Eine Beschattung steigerte die Gesamtgehalte an P und K in den jungen Teezweigen. Die reduzierten Aktivitäten der Nitratreduktase (NR) und der Phenylalanine ammonium-lyase (PAL) unter Beschattung hemmten die Synthese der Vorstufen der Catechine. Weiterhin mag die gesteigerte Polyphenoloxidase-aktivität (PPO) unter Beschattung die Degradation von Polyphenolen verstärkt haben. Die Kombination dieser Prozesse kann teilweise erklären, warum eine Beschattung die TP/AA senken konnte und mithin die Qualität von grünem Tee verbesserte. Unter Beschattung wurde ein gesteigerter Blattwassergehalt und eine erhöhte spezifische Blattfläche sowie ein geringeres Chlorophyll a/b-Verhältnis und ein verminderter Gesamt-N-Gehalt pro Einheit Blattfläche als typische Anpassungen der Teepflanze an niedrige Einstrahlung identifiziert, um mehr Lichtenergie pro Einheit Biomasse einzufangen. Eine gesenkte Lichtsättigungsrate ( $A_{max}$ ), ein niedrigerer Lichtkompensationspunkt ( $I_c$ ) und eine verminderte Dunkelatmungsrate ( $R_d$ ) unter

Beschattung erklärten teilweise, warum eine Beschattung den Ertrag vermindert hat. Gesenkte Aktivitäten der Ascorbatperoxidase (APX), der Dehydroascorbatreductase (DHAR), der Glutathionreductase (GR) und verminderte Gehalte an Vitamin C unter Beschattung implizieren, dass die lichtgesteuerte Aufbau des antioxidativen Potentials bei Beschattung weniger ausgeprägt ist.

Anfänglich wurde die Eignung verschiedener Schnelltestmethoden wie SPAD-Meter, Nitratelektrode und die reflektometrische Nitratbestimmung verglichen. Die Nitratbestimmung mittels Reflektometer ( $\text{NO}_3^-$ -Teststreifen) erwies sich als am besten geeignet, um den  $\text{NO}_3^-$ -Gehalt im Presssaft des Teegewebes zu messen. Die Ninhydrinmethode wurde verwendet, um die Gehalte an Aminosäuren-N (alpha-Amino-N, basierend auf dem Standard Glutaminsäure) im Teepresssaft zu messen. 95% des maximalen Ertrages wurden vor dem Hintergrund der Gewinnmaximierung als optimaler Ertrag angesehen. Die optimale N-Applikationsrate ( $N_{\text{opty}}$ ), entsprechend 95% des Maximalertrags betrug  $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  und lag nahe dem N-Niveau, welches die niedrigsten TP/AA-Verhältnisse (Verhältnis von Polyphenolen zu Aminosäuren, das invers mit der Qualität von Grüntee korreliert ist) ergab. Im Frühling lagen die kritischen  $\text{NO}_3^-$ -Gehalte in unterschiedlichen Organen bei jeweils  $30\text{-}33 \text{ mg L}^{-1}$  in jungen Sprossachsen,  $6\text{-}7 \text{ mg L}^{-1}$  in den voll entfalteten neuen Blättern (NFEL) und  $10\text{-}11 \text{ mg L}^{-1}$  in den obersten voll entwickelten Blättern (Die Blätter der braunen Sprossachsen stammten aus dem vorherigen Jahr). Der kritische AAN-Gehalt betrug  $714\text{-}732 \text{ mg L}^{-1}$  in NFEL. Im Herbst wurden als kritische Gehalte der Indikatoren  $5\text{-}6 \text{ mg L}^{-1} \text{ NO}_3^-$  und  $495\text{-}510 \text{ mg L}^{-1} \text{ AAN}$  in den voll entwickelten Blättern (ML) ermittelt. Die kritischen Boden- $N_{\text{min}}$ -Werte in 0-60 cm Bodentiefe betragen jeweils  $200\text{-}260 \text{ kg N ha}^{-1}$  im Frühling und  $320\text{-}430 \text{ kg N ha}^{-1}$  im Herbst. Die optimale N-Applikationsraten innerhalb einer Erntesaison ( $N_{\text{opts}}$ ) waren jeweils  $240\text{-}340 \text{ kg N ha}^{-1}$  im Frühling und  $130\text{-}240 \text{ kg N ha}^{-1}$  im Herbst. Im Sommer hatte die Beschattung einen starken Effekt auf  $N_{\text{opty}}$  ( $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  in voller Sonne und  $700 \text{ kg N ha}^{-1} \text{ a}^{-1}$  unter Beschattung) und die kritische  $\text{NO}_3^-$ -Konzentration in jungen Sprossachsen ( $20\text{-}33 \text{ mg L}^{-1}$  in voller Sonne und  $32\text{-}51 \text{ mg L}^{-1}$  unter Beschattung). Aber eine Beschattung hatte keinen Effekt auf die kritischen Boden- $N_{\text{min}}$ -Werte ( $180\text{-}230 \text{ kg N ha}^{-1}$  unter beiden Bedingungen, beschattet und exponiert) und  $N_{\text{opts}}$  ( $140\text{-}240 \text{ kg N ha}^{-1}$  unter beiden Bedingungen, beschattet und exponiert). Basierend auf einer optimalen N-Applikationsrate von  $810 \text{ kg N ha}^{-1} \text{ a}^{-1}$  betrug bisher die Aufteilung der N-Applikationsraten im Frühling (30%) und Herbst (20%) jeweils  $240 \text{ kg N ha}^{-1}$  und  $160 \text{ kg N ha}^{-1}$ . Wegen des starken Effekts einer Beschattung auf  $N_{\text{opty}}$  ( $810 \text{ kg}$

$\text{N ha}^{-1} \text{ a}^{-1}$  in voller Sonne und  $700 \text{ kg N ha}^{-1} \text{ a}^{-1}$  unter Beschattung), entsprechend 20% Niveau  $160 \text{ kg N ha}^{-1}$  bzw.  $140 \text{ kg N ha}^{-1}$ . Daher sollte der Anteil der N-Applikationsrate zu den drei Ernteperioden nach der vorliegenden Untersuchung angepasst werden. Bei der Frühlingsdüngung lagen die N-Applikationsraten im unteren Bereich des Schwellenwertes von  $N_{\text{opts}}$  ( $240\text{-}340 \text{ kg N ha}^{-1}$ ), während für den Tee im Herbst die N-Applikationsrate im Bereich von  $N_{\text{opts}}$  ( $130\text{-}240 \text{ kg N ha}^{-1}$ ) erfolgte. Wird bei der Sommerernte von Grüntee der für beschattete Teesträucher ermittelte Grenzwert zugrunde gelegt, ist die N-Applikation nicht ausreichend, weil die applizierte N-Menge im Sommer lediglich im Bereich des unteren begrenzenden Schwellenwertes ( $N_{\text{opts}}$ ,  $140\text{-}240 \text{ kg N ha}^{-1}$ ) lag. Daher kann nach dieser Untersuchung die Applikationsrate im Herbst reduziert werden und sollte zu dem im Frühjahr und Sommer geerntetem Tee ausgebracht werden, insbesondere aber im Frühjahr.

Die Applikation von Kalium- und Magnesiumdüngern konnte den Ertrag steigern und die Qualität verbessern, insbesondere bei der Sorte Longjing43 in 2003. Wegen des Antagonismus von K und Mg war der Effekt von K auf eine Steigerung des Gehalts an Aminosäuren und des Quotienten von Ascorbinsäure zu Dehydroascorbinsäure stärker ausgeprägt als bei gemeinsamer Anwendung von K und Mg. Währenddessen waren der gesteigerte Gehalt an austauschbarem Ca und der Antagonismus von Ca, K und Mg für den begrenzten Effekt einer gemeinsamen Anwendung von K und Mg hinsichtlich einer Ertrags- und Qualitätssteigerung verantwortlich.

Eine Schwefelapplikation konnte den Frischmasseertrag, sowie den Gehalt an freien Aminosäuren und Gesamtascorbinsäure leicht steigern. Die Polyphenolgehalte und der TP/AA Quotient waren hingegen vermindert. Währenddessen konnte die Applikation von Schwefel das von N:S-Verhältnis leicht senken. Die Aktivitäten von NR und PAL waren erhöht, wohingegen die Aktivität von PPO vermindert war. Die relativ gute Schwefelversorgung des Standortes verhinderten stärkere Auswirkungen der S-Zufuhr auf Ertrag und Qualität.



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## Appendix

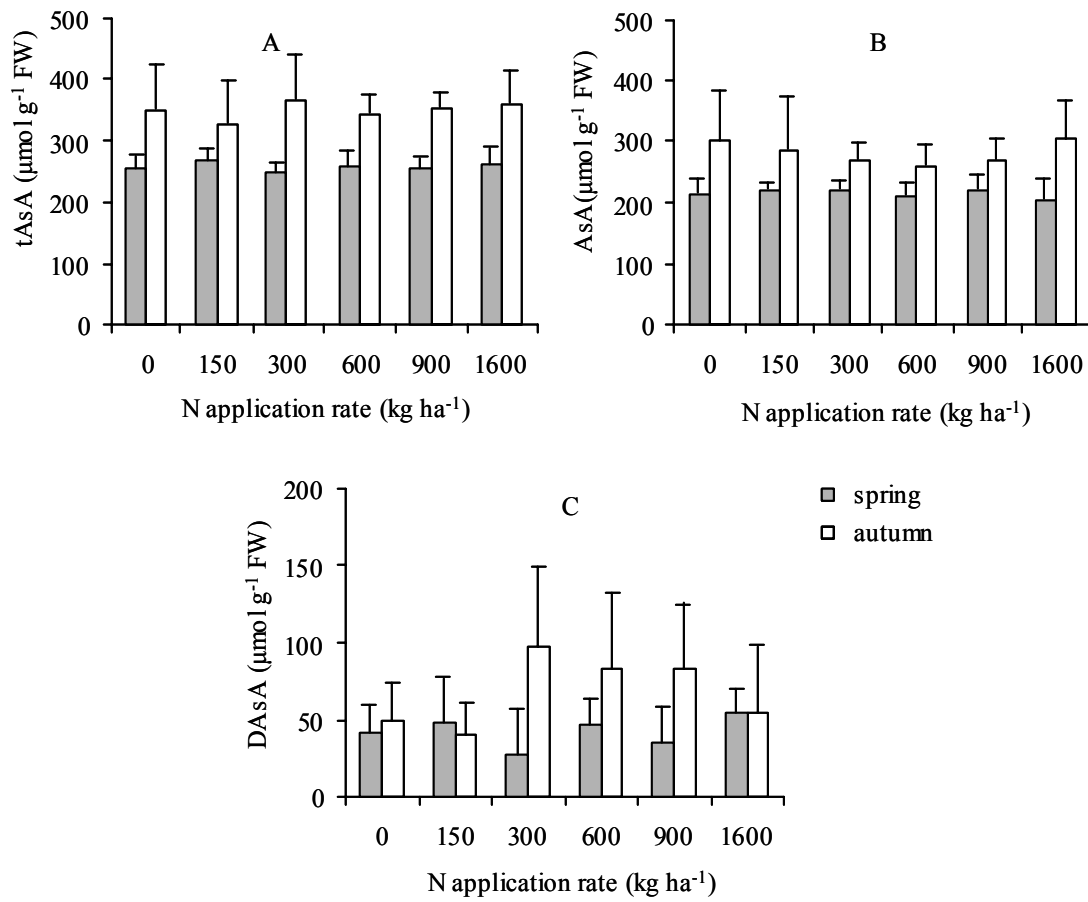


Fig. A1 The effect of N levels on the contents of total ascorbic acid (tAsA), ascorbic acid (AsA) and dehydroascorbic acid (DAsA) of tea flushes in spring and autumn in 2003.

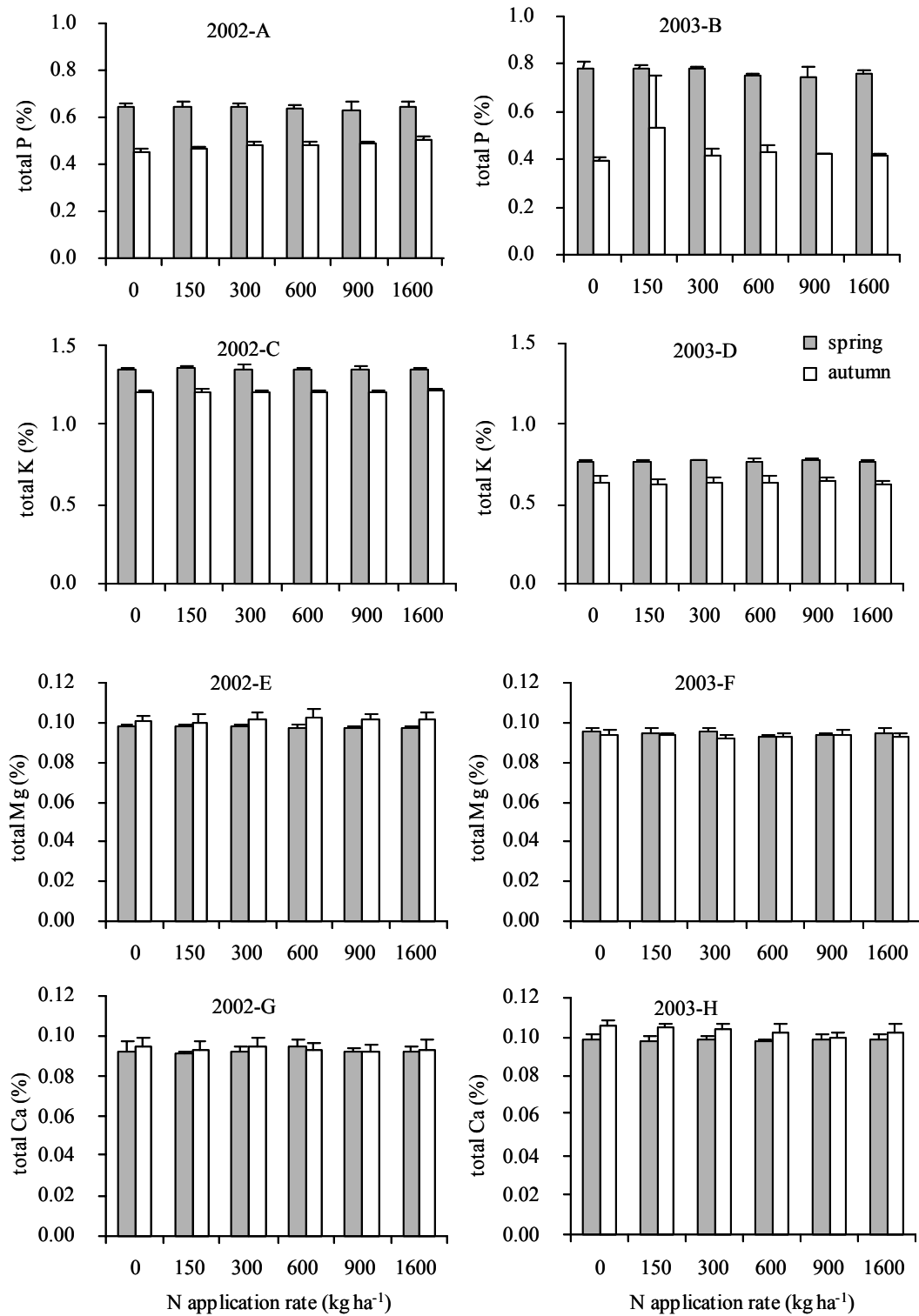


Fig. A2 The effect of N levels on the total contents of P, K, Mg and Ca in green tea flushes in both years.

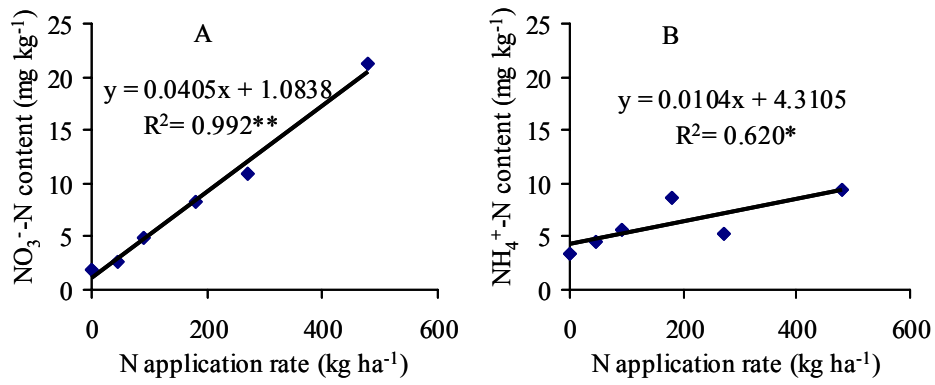


Fig. A3 The relationship between nitrogen application rate and the content of nitrate and ammonium nitrogen in 0-30 cm soil in spring of 2003. 30% of N for each treatment was applied on February 25 and the soil samples were taken on April 22.

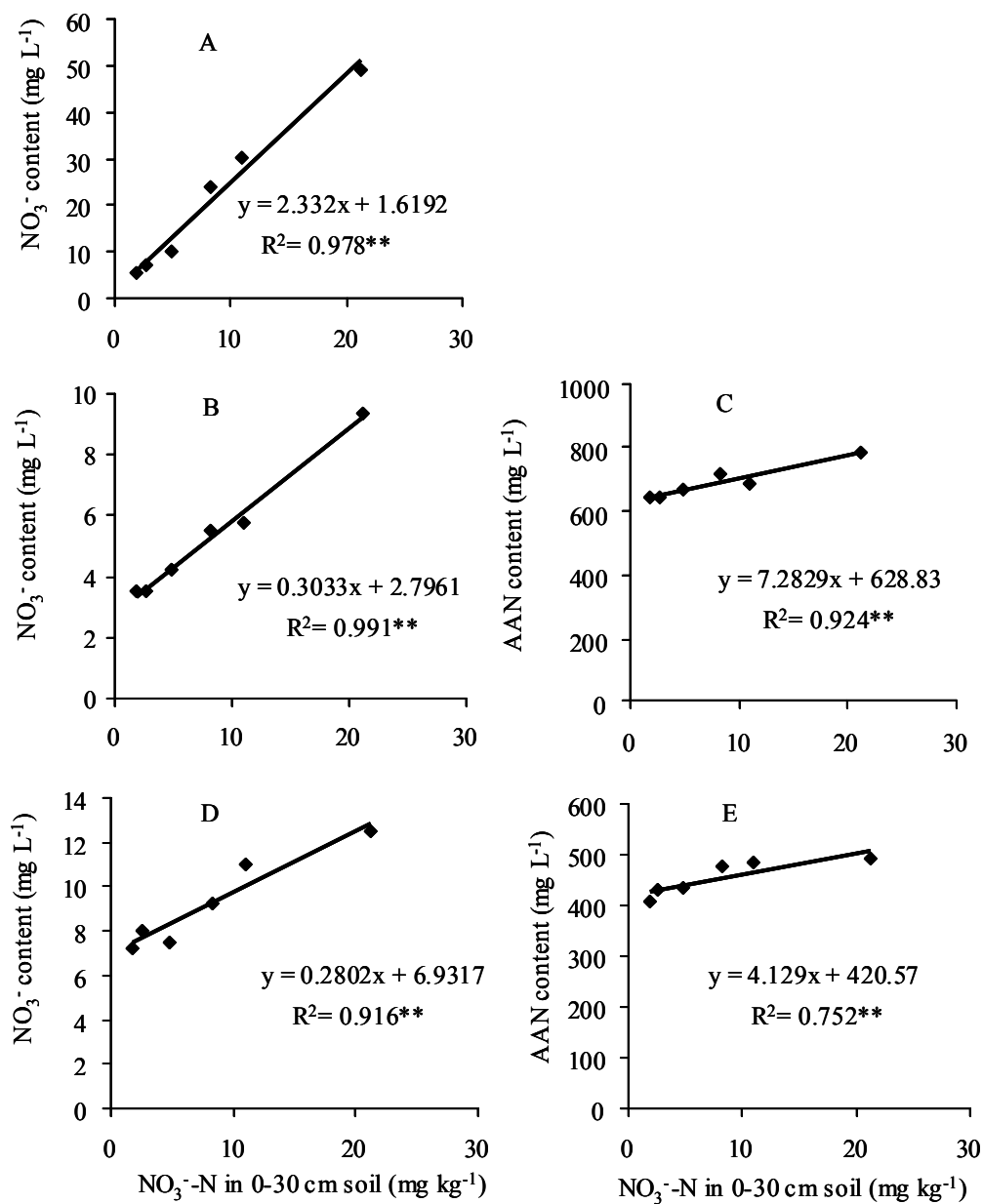


Fig. A4 The relationship between nitrate in young stem, new fully expanded leaves, top old leaves and nitrate nitrogen in 0-30 cm soil in spring of 2003. (A: nitrate in the young stem; B, C: nitrate and amino acid nitrogen (AAN) in new fully expanded leaves; D, E: nitrate and amino acid nitrogen (AAN) in the top old leaves which were on the brown stems that grew up from the former year).

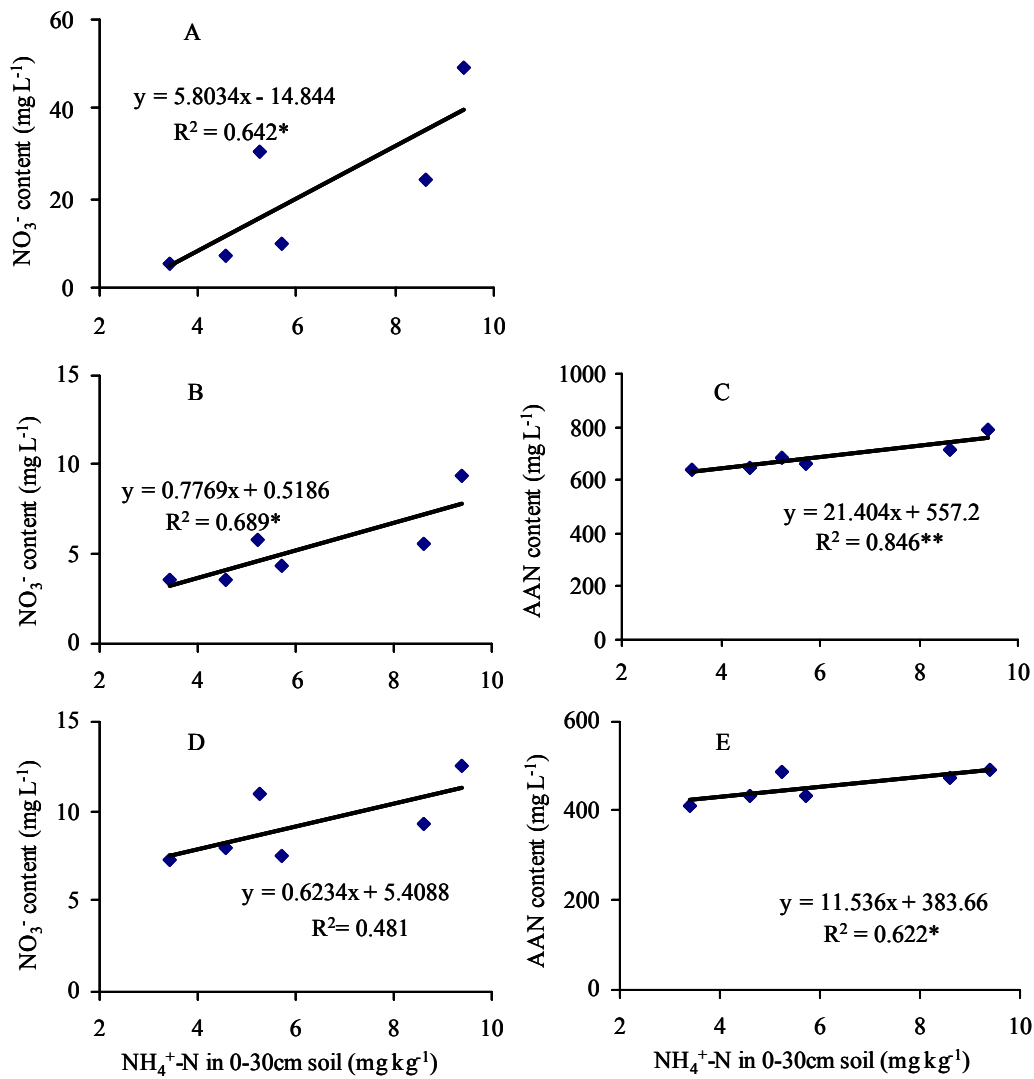


Fig. A5 The relationship between nitrate in young stem, new fully expanded leaves, top old leaves and ammonium nitrogen in 0-30 cm soil in spring of 2003. (A: nitrate in the young stem; B, C: nitrate and amino acid nitrogen (AAN) in new fully expanded leaves; D, E: nitrate and amino acid nitrogen (AAN) in the top old leaves which were on the brown stems and grew up from the former year).

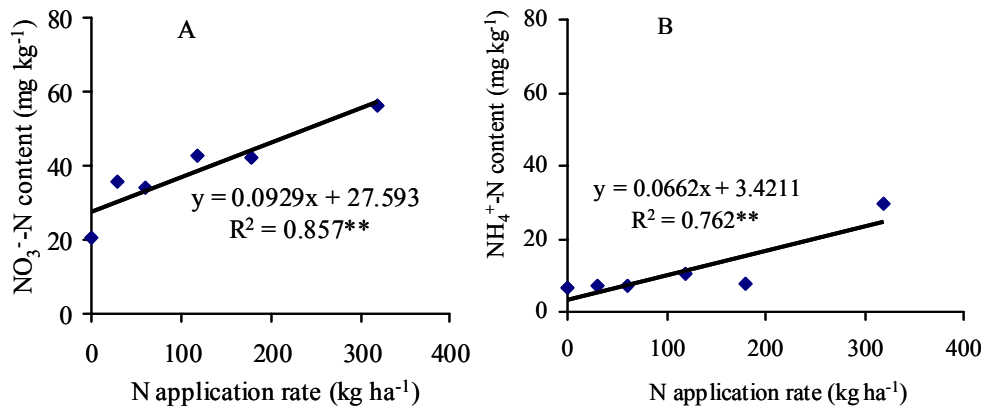


Fig. A6 The relationship between nitrogen application rate and nitrate nitrogen and ammonium nitrogen in 0-30 cm soil in autumn of 2003. 20% of N for each treatment was applied on August 22 and the soil samples were taken on September 20.

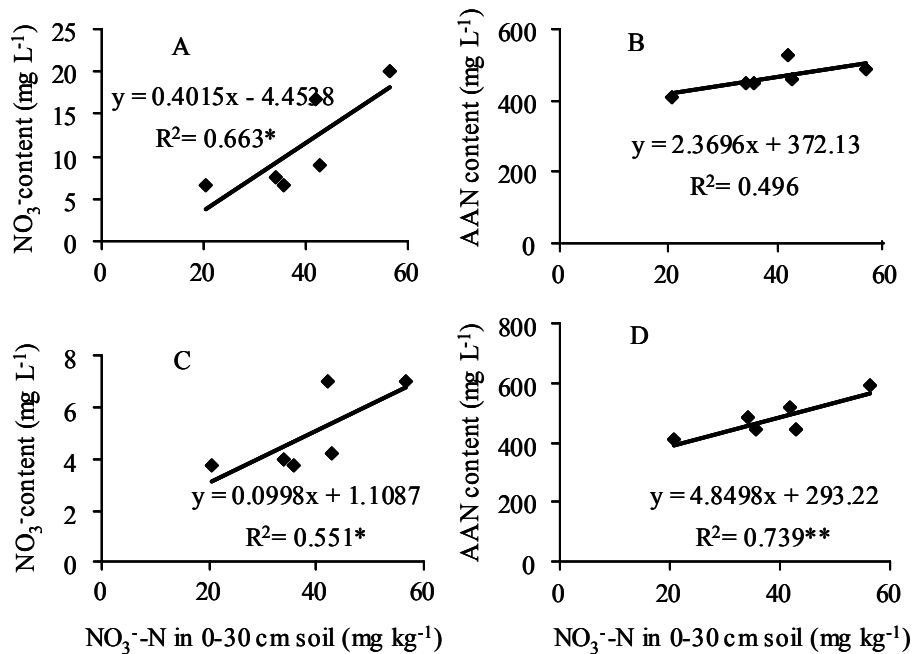


Fig. A7 The relationship between nitrate and amino acid nitrogen in young stem and mature leaves and nitrate nitrogen in 0-30 cm soil in autumn of 2003. (A, B: nitrate content and amino acid nitrogen (AAN) in young stem; C, D: nitrate content and amino acid nitrogen (AAN) in mature leaves).

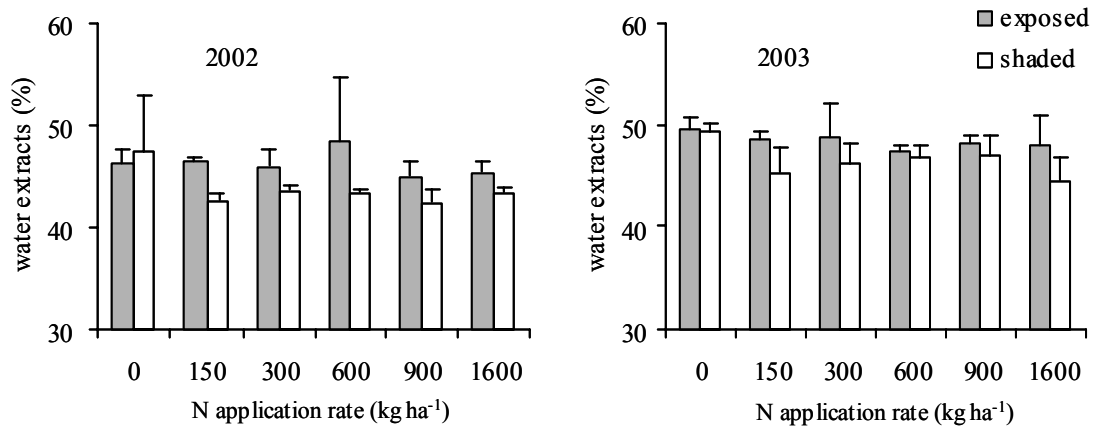


Fig. A8 Effect of the interaction between light intensity and nitrogen application rate on water extract in tea flushes (one bud and two leaves) after shading for 15 days in summer of 2002 and 2003.

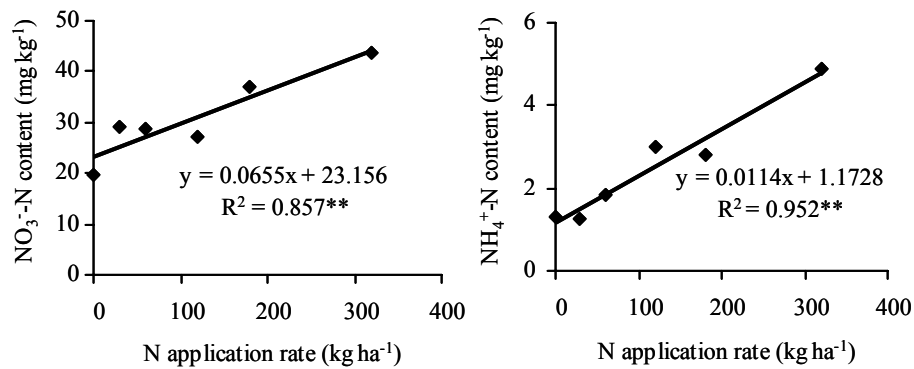


Fig. A9 The relationship between NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N in 0-30 cm soil and N application rate in summer of 2003. 20% of N for each treatment was applied after pruning on May 23 and the soil samples were taken on August 13 after shading for 15 days.

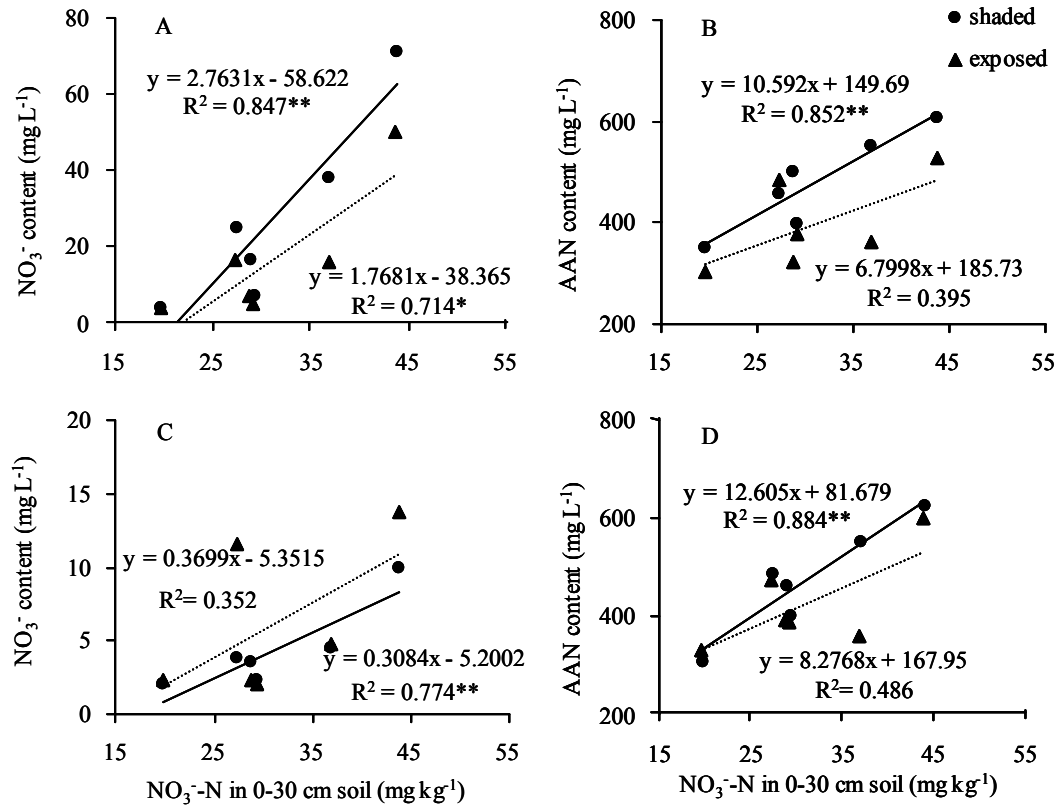


Fig. A10 The relationships between the contents of nitrate ( $\text{NO}_3^-$ ) and amino acid nitrogen (AAN) in young stem and in mature leaves and  $\text{NO}_3^-$ -N in 0-30 cm soil under shaded and exposed conditions (A, B:  $\text{NO}_3^-$  and AAN in young stem; C, D:  $\text{NO}_3^-$  and AAN in mature leaves).

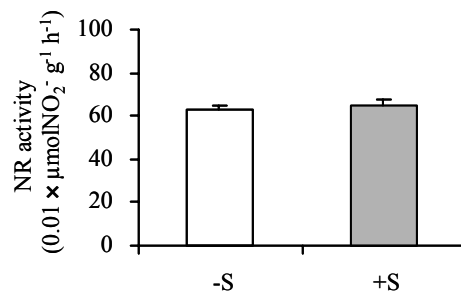


Fig. A11 The effect of sulphur on the activity of nitrate reductase (NR) in the 1<sup>st</sup> leaf in the tea flushes (one bud and one leaf) in summer of 2003.



Table A1 The effect of sulphur on the components of amino acids (AA,  $\mu\text{mol g}^{-1}$  dry weight) in spring tea flushes in 2002 and 2003.

AA	2002		2003	
	-S	+S	-S	+S
Asp	7.700a <sup>§</sup>	7.765a	6.758a	6.718a
Thr	0.813a	0.955b	0.955a	0.901a
Ser	3.980a	4.418b	4.179a	3.996a
Asn	0.330a	0.402b	0.313a	0.372a
Glu	10.375a	11.573a	9.230a	8.955a
Gln	14.850a	16.615a	14.220a	14.157a
Theanin	43.158a	46.550a	49.942a	47.754a
Gly	0.458a	0.680a	0.818a	0.842a
Ala	3.000a	4.345a	3.263a	3.233a
Val	2.483a	2.663a	2.453a	2.473a
Leu	0.260a	0.255a	0.204a	0.294a
Tyr	0.378a	0.295a	0.286a	0.305a
Phe	0.380a	0.337a	0.446a	0.462a
GABA	4.943a	5.045a	4.304a	4.494a
His	0.283a	0.263a	0.374a	0.344a
Arg	0.838a	1.548b	0.992a	0.954a
Total	94.431a	103.777a	98.944a	96.468a

<sup>§</sup>: numbers followed by same small letters indicate the treatment means are not significantly different at  $p < 0.05$  ( $n=4$ ).



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