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茶园施肥模式对茶叶黄酮类及糖苷类代谢物含量的影响

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摘要:【目的】研究施肥模式对茶叶生化成分含量和黄酮类及糖苷类代谢物累积的影响，为实现茶叶优质高产提供科学依据。【方法】田间试验设在四川茶树种植典型区域—雅安，试验连续进行了 3 年，设置不施肥 (CK)、常量化肥 (N 585 kg/hm^2，TF)、有机肥替代 25% 化肥氮 (OF)、减施 25% 氮肥 (SF) 4 个处理。分析了不同处理茶叶主要品质成分含量，并基于非靶向代谢组学检测结果分析了黄酮类及糖苷类代谢物累积差异。【结果】与 CK 处理相比，TF、OF、SF 处理茶氨酸、游离氨基酸含量分别提高了 7.22%～13.40%、23.15%～25.50%，可溶性糖和茶多酚含量分别降低了 8.17%～13.86%、6.08%～11.49%，表儿茶素、表没食子儿茶素、芸香苷等 10 种代谢物累积水平显著下降。与 TF 处理相比，SF 处理茶氨酸、咖啡碱和水浸出物含量分别降低了 5.45%、5.97%、8.91%，茶多酚、可溶性糖、游离氨基酸含量和酚氨比无显著差异；芍药素-3-O-葡萄糖苷、肉桂萜醇 C1-19-葡萄糖苷、查耳酮樱花素等 7 种代谢物累积水平显著降低，川陈皮素、牡荆素-4'-O-α-L-吡喃鼠李糖苷、(S)-橙花醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] 和 Ranupenin3-rutinoside 等 4 种代谢物累积水平显著增加。与 TF 处理相比，OF 处理茶叶咖啡碱含量降低了 5.73%；茶氨酸、游离氨基酸、可溶性糖、茶多酚、水浸出物含量差异不显著；10 种黄酮苷、2 种黄酮、6 种萜苷、5 种甾体皂甙和 2 种脂酰苷共 25 种代谢物累积水平显著增加，以川陈皮素、飞燕草素-3-(6-对香豆酰基半乳糖苷) 增幅较大，分别为 2280.20%、1355.11%。4 个处理相比，OF 处理下有 15 种黄酮类及糖苷类代谢物累积水平显著高于 CK、TF 和 SF 处理。【结论】有机肥替代 25% 化肥氮降低了茶叶咖啡碱含量，提高了川陈皮素、飞燕草素、槲皮素、芹黄素、山奈酚、牡荆素等多种黄酮类代谢物和去氢大豆皂甙 I、珠子参苷 R2、孕甾烷甙等多种糖苷组分累积水平。减施 25% 氮肥降低了茶氨酸、咖啡碱、水浸出物含量和糖苷类代谢物 (如杨梅酮-3-O-洋槐糖苷、芍药素-3-O-葡萄糖苷、肉桂萜醇 C1-19-葡萄糖苷等) 累积水平，对茶多酚、可溶性糖、游离氨基酸含量影响较小。有机肥替代部分化肥氮有利于茶叶黄酮类及糖苷类物质累积，单纯减少氮肥投入会降低茶叶品质。

关键词: 施肥；有机肥替代化肥；氮肥减施；黄酮；糖苷；茶叶品质

Effects of fertilization patterns on flavonoids and glycoside metabolites in tea

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Abstract:【**Objectives**】This study examines the effects of fertilization patterns on the biochemical components and the accumulation of flavonoids and glycoside metabolites in tea leaves.【**Methods**】The 3-year field experiment was conducted in Ya'an which was a typical region of tea plant cultivation in Sichuan Provence, consisted of four treatments: no fertilization (CK), conventional chemical fertilizer input (N 585 kg/hm^2-TF), 25%

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replacement of chemical fertilizer N with organic manure (OF), and reducing 25% of N supply (SF). The content changes in main quality components of tea leaves with different fertilization patterns were analyzed, as well as the accumulation variations of flavonoids and glycosidic metabolites based on the results of untargeted metabolomics. 【**Results**】Compared with CK treatment, the contents of the anine and free amino acids in tea leaves under TF, OF, and SF treatments were significantly increased by 7.22%–13.40%, 23.15%–25.50%, respectively, while soluble sugar and tea polyphenols contents decreased by 8.17%–13.86%, 6.08%–11.49%, respectively. Concurrently, accumulation levels of 10 metabolites including epigallocatechin, epicatechin, and rutin decreased notably. Compared with TF treatment, the contents of theanine, caffeine and water-soluble extracts in tea leaves under SF treatment significantly decreased by 5.45%, 5.97%, 8.91%, respectively, while the content of tea polyphenols exhibited no significant difference as well as soluble sugar and free amino acid content. In addition, accumulation levels of 7 metabolites (e.g. peonidin 3-O-glucoside, cinncassiol C1 19-glucoside and chalconosakuranetin) decreased markedly, while that of 4 metabolites [e.g. nobiletin, vitexin 4'-O-α-L-rhamnopyranoside, (S)-Nerolidol 3-O-[a-L-rhamnopyranosyl-(1->2)-β-D-glucopyranoside] increased significantly. The contents of caffeine in tea leaves with OF treatment was 5.73% lower than that in TF. However, accumulation levels of 25 metabolites including 10 kinds of flavonoid glycosides, 2 kinds of flavone, 6 kinds of terpene glycosides, 5 kinds of steroidal glycosides, and 2 kinds of fatty acyl glycosides compounds increased significantly, especially nobiletin and delphinidin 3-(6-p-coumaroylgalactoside) showed the higher increase at 2280.20% and 1355.11%, respectively. Among the four fertilization treatments, 15 kinds of flavonoids and glycosides metabolites demonstrated higher accumulation levels in tea leaves with OF treatment compared with CK, TF, and SF treatments.【**Conclusions**】Replacing 25% of chemical fertilizer N with organic manure reduced the content of caffeine in tea leaves, and improved the accumulation levels of a variety of flavonoids metabolites (e.g. nobiletin, delphinidin, quercetin, apigenin, kaempferol, vitexin) and glycosylic components (e.g. dehydrosoyasaponin I, majonoside R2, balagyptin). Reducing 25% of N supply decreased the contents of theanine, caffeine and water-soluble extracts as well as the accumulation levels of glycosylic components (e.g. myricetin 3-O-robinobioside, peonidin 3-O-glucoside, cinncassiol C1 19-glucoside), and had less effect on tea polyphenols, soluble sugar, and free amino acid content. Partial substitution for chemical fertilizer N with organic manure is beneficial for the accumulation of flavonoids and glycosides in tea leaves. Onefold reduction of N input will reduce the quality of tea.

Key words: fertilization; organic manure substitution for chemical fertilizer; reduction of N supply; flavonoids; glycosides; tea quality

茶树是我国重要的经济作物之一，茶叶因其独特的风味口感和人体保健价值正成为仅次于水的世界第二大饮品[1]。茶鲜叶中的多酚类、氨基酸、咖啡碱、可溶性糖等天然产物是加工形成优质茶叶产品的物质基础[2–3]，其含量高低及配比主要由茶树品种决定[4–5]，同时受土壤、气候等茶园环境[6–10]和栽培措施影响[11–14]。施肥直接影响茶园土壤养分含量及肥力，进而影响茶树对养分的吸收利用和茶叶生化品质，对茶鲜叶综合品质差异的贡献度约为 7.48%[15]。氮是茶树营养“三要素”之一，过量施氮或施氮不足均不利于茶树生长和茶叶品质，且过量施肥加重环境负荷，造成面源污染[16–18]。因此，茶园养分管理以氮肥投入为核心，大量研究围绕“茶园适宜供氮”展开。有研究提出茶园年施氮肥 300～450 kg/hm^2 [19]，最有利于茶鲜叶品质维持在较高水平；刘美雅等[20]研究表明茶园年施氮 285～474 kg/hm^2，茶叶氨基酸、β-芳樟醇、橙花叔醇含量显著增加；刘扬等[15]提出福建省安溪县铁观音茶园高产优质的适宜施氮量为每年 200～400 kg/hm^2。Sun 等[21]研究表明，分别以有机肥 (牛粪) 和尿素施入等量氮养分，茶叶中氨基酸、糖、脂肪酸的富集途径差异明显，牛粪处理使谷氨酰胺、奎宁酸和脯氨酸积累较多，而十八烷酸、十六烷酸和二十烷酸等有机酸含量显著减少，所制绿茶感官品质更优；Xie 等[22]建议用有机肥氮替代 20% 化肥氮；朱旭君等[23]研究认为有机肥替代化肥氮的比例在 50% 以上更有利于提高茶园产量

和名优绿茶品质。Tang 等[24]研究表明，铵态氮较硝态氮更易被茶树吸收利用。Li 等[25]鉴定出 *AMT*、*NRT*、*AQP* 是调控茶树氮吸收的关键基因，氮累积则受 *GOGAT* 和 *GS* 等基因控制，*CHS*、*CHI*、*DFR* 与儿茶素合成相关[26]，不同供氮量和氮形态均会诱导相关基因差异表达。

黄酮类化合物是茶叶多酚物质的重要组分[27]，约为茶多酚含量的 13%，占茶叶 (干重) 的 3%～4%，是茶叶“苦涩、收敛”风味和保健功能的重要物质来源[1]。茶叶中的黄酮类代谢物主要有黄烷醇、花色素、黄酮和黄酮醇等[28]，除黄烷醇外，多以黄酮糖苷形式存在，如芸香苷、槲皮苷、山奈苷、花色苷等[29]，是绿茶汤色“黄绿”的构成因子。除黄酮苷外，茶鲜叶中还存在其它糖苷类代谢物，如萜苷、皂甙、脂酰苷等，在内源糖苷酶作用下释放出单萜烯醇或芳香族醇等具有愉快花果香的游离态苷元，是茶叶香气的主要前体物质[30]。不同茶叶品类[31–33]、不同茶树品种[5]生化成分差异明显，因而品质各异。茶叶中黄酮类代谢物的积累模式和调控机制随不同氮素条件而存在显著差异，Huang 等[34]茶树幼苗水培试验表明，缺氮促进多种黄酮类物质累积，随着氮供应量增加，黄酮类物质合成受到抑制。Dong 等[35]茶树幼苗盆栽试验表明，茶叶中槲皮素-3-葡萄糖苷、山奈素-3-半乳糖苷等多种黄酮苷与蔗糖、果糖、葡萄糖含量呈显著正相关，适量供氮诱导黄酮和碳水化合物代谢相关基因上调表达，从而促进黄酮苷合成，氮过量或缺氮均表现为抑制作用。Liu 等[36]研究指出，茶树嫩梢黄酮类物质的累积与茶树根系、成熟叶片中的碳氮代谢密切相关，增加氮供应促进茶树树体碳向成熟叶和根系分配，导致嫩叶中用于黄酮类物质合成的“碳骨架”减少。就氮形态而言，硝态氮促进茶多酚、儿茶素合成相关基因表达和累积量增加，铵态氮促进茶氨酸、谷氨酸、精氨酸累积量增加[37]。此外，茶树黄酮类物质还受植物激素调控，如孙平[38]研究发现，脱落酸 (ABA) 处理后茶树中苯丙烷途径和类黄酮途径的基因 (*DHD*、*SDH1* 等) 表达均显著上升。

然而，茶园栽培条件下，长期不同施氮方式对茶叶黄酮类及糖苷组分的影响鲜有研究。这些物质不仅是茶叶的重要品质成分，还能为深入了解茶树对环境变化的生理响应提供重要信息[39–40]。本研究在四川茶树种植的典型区域，通过连续 3 年田间试验比较了不施肥、常量化肥氮、有机肥替代和减施氮肥对茶叶生化品质的影响，并基于非靶向代谢组学检测结果，分析了不同施氮模式下茶叶黄酮类及糖苷类代谢物的累积差异，旨在丰富茶园养分高效管理及绿色生产的理论基础，为实现茶叶优质高产提供科学依据。

1　材料与方法

1.1　试验地概况

试验地位于四川省农业科学院茶叶研究所国家土壤质量雅安观测实验站基地 (30°16′N、103°17′E，海拔 766 m)，属于亚热带季风性湿润气候，年平均气温 15.8℃，无霜期 297 天，年降雨量 1500 mm 左右。茶园土壤类型为酸性黄壤土，0—20 cm 土层土壤基础理化性质为：pH 4.42、有机质 17.5 g/kg、全氮 0.97 g/kg、全磷 0.38 g/kg、全钾 13.81 g/kg、硝态氮 9.51 mg/kg、铵态氮 0.57 mg/kg、有效磷 12.7 mg/kg、速效钾 147 mg/kg；20—40 cm 土层土壤基础理化性质为：pH 4.80、有机质 12.3 g/kg、全氮 0.79 g/kg、全磷 0.37 g/kg、全钾 12.15 g/kg、硝态氮 5.80 mg/kg、铵态氮 1.48 mg/kg、有效磷 13.4 mg/kg、速效钾 92.0 mg/kg。

1.2　试验设计

试验设 4 个处理：1) 不施肥对照 (CK)；2) 常量化肥氮 (TF)；3) 与 TF 施氮总量一致，以有机肥氮替代 25% 化肥氮 (OF)；4) 采用茶树专用配方肥，氮肥减量 25% 并增施钾肥 (SF)。常量化肥氮施用量为 N 585 kg/hm^2，试验用氮磷钾复合肥的 N–P_2O_5–K_2O 为 15%–15%–15%)，尿素含 N 46.3%。供试有机肥为商品有机肥 (N–P_2O_5–K_2O 3.2%–0.8%–1.0%) 和茶树专用配方肥 (N–P_2O_5–K_2O 22%–9%–15%)。每年 10 月中旬开沟基施肥料，沟深 20 cm，翌年春茶结束一次性撒施追施。每个处理设 3 次重复，共设 12 个试验小区，小区面积约 400 m^2，随机排列，不同小区间设 2 行隔离茶行。各处理病虫害防治、茶园采摘、修剪等其它管理措施一致。试验期为 2016—2018 年，各处理施肥量及年养分投入见表 1。

1.3　样品采集

2019 年春季，于茶树蓬面新梢 30% 通过一芽二叶物候期采摘一芽二叶鲜叶，随机称取各处理鲜叶 500 g，采用蒸青固样、自然阴干[41–42]，用于茶叶主要品质成分测定；另取 100 g 鲜叶立即放入液氮保存，用于 LC-MS 非靶向代谢检测。

1.4　测定项目与方法

1.4.1　光合生理指标测定　　于天气晴朗、无风少云

表1 不同施肥处理年养分投入 (kg/hm^2)

Table 1 Annual nutrient inputs of fertilization treatments in field experiment

处理 Treatment	肥料用量 Fertilizer dosage		养分投入量 Nutrient dosage				
	基肥 Basal fertilizer	追肥 Topdressing fertilizer	无机氮 Inorganic N	有机氮 Organic N	总氮 Total N	P_2O_5	K_2O
CK	0	0	0	0	0	0	0
TF	1125 (氮磷钾复合肥 Compound fertilizer)	900 (尿素 Urea)	585	0	585	169	169
OF	4575 (商品有机肥 Commercial organic fertilizer) + 844 (氮磷钾复合肥 Compound fertilizer)	675 (尿素 Urea)	439	146	585	163	172
SF	2000 (茶树专用配方肥 Specific formulated fertilizer for tea tree)	0	440	0	440	180	300

注：CK—不施肥处理；TF—常规化肥处理；OF—有机肥氮替代25%化肥氮处理；SF—减施25%化肥氮处理。

Note: CK—No fertilizer; TF—Conventional chemical fertilizer input; OF—Replacing 25% of chemical fertilizer N with organic N; SF—Reducing 25% of chemical fertilizer N.

之日，采用 Li-COR 6800 型植物光合测量系统进行光合生理指标采集。选取茶树蓬面向光性良好的新梢成熟叶片 (第四片或第五片)，每个处理测定 20 个叶片 (重复)、每个叶片采集 10 组数据，测定时间为 2019 年 4 月 26 日上午 9：30—11：00。

1.4.2 茶叶品质成分含量测定方法 1) 茶多酚含量测定参照 GB/T 8313—2018，采用分光光度法；游离氨基酸含量测定参照 GB/T 8314—2013，采用茚三酮比色法；咖啡碱含量测定参照 GB/T 8312—2013，采用高效液相色谱法；水浸出物含量测定参照 GB/T 8305—2013，采用差量法；可溶性糖总量测定采用蒽酮比色法；茶氨酸含量测定参照 GB/T 23193—2017，采用高效液相色谱法。酚氨比为茶多酚含量与游离氨基酸含量之比。

2) 黄酮类及糖苷类代谢物测定，称取经液氮研磨的茶鲜叶样品 50 mg，加入 400 μL 提取液 [*V*(甲醇):*V*(水)=4∶1]。采用高通量组织破碎仪低温破碎 (–20℃，50 HZ，6 min)。涡旋 (30 s) 混匀后，低温超声萃取 30 min (5℃，40 KHz)。将样品静置 (–20℃，30 min) 后离心 (13000 g、4℃、15 min)，取上清液并转移至 LC-MS 进样小瓶中上机分析。

采用超高效液相色谱串联飞行时间质谱系统 (AB SCIEX, UHPLC-Triple TOF) 进行非靶向代谢物检测[31]。色谱条件：色谱柱为 BEH C18 柱 (100 mm × 2.1 mm i.d., 1.7 µm; Waters, Milford, USA)；流动相 A 为去离子水 (含 0.1% 甲酸)，流动相 B 为乙腈/异丙醇 (1/1) (含 0.1% 甲酸)；进样量 10 µL，流速为 0.40 mL/min，柱温 40℃。流动相洗脱梯度：0～3 min，95% A、5% B；3～9 min，80%～5% A、20%～95% B；保持 4 min；13～16 min，95% A、5% B。质谱条件：分别采用正、负离子扫描模式，离子喷雾电压 (+5 kv、–4 kv)、喷雾气 50 psi、辅助加热气 50 psi、离子源加热温度 500℃、质量范围 50～1000 M/Z、碰撞电压 20～60 eV。

分析系统的稳定性采用质控样品 (QC) 进行考察。质控样品由所有检测样品混合而成，在仪器分析过程中，每 3 个样品插入 1 个质控样本，通过该样本的重复性分析考察检测过程中仪器稳定性，同时筛查分析系统中变异较大的变量，保证结果的可靠性。

3) 黄酮类及糖苷类代谢物的鉴定，首先，将 UPLC-Q-TOF/MS 分析得到的原始数据导入代谢组学处理软件 Progenesis QI (Waters Corporation，Milford, USA)，进行基线过滤、峰识别、积分、保留时间校正、峰对齐，得到保留时间、质荷比和峰强度等质谱信息，并与代谢数据库 (http://www.hmdb.ca/；https://metlin.scripps.edu) 进行匹配，对检测到的代谢物定性。然后，通过保留至少一组样品中非零值 80% 以上的变量、原始矩阵极小值补缺、总峰归一化、删除质控样本相对标准偏差 (RSD)≥30% 的变量等数据预处理，去除搜库得分<50.0 的代谢物。最后，依据 HMDB 数据库中物质分类及物质结构式筛选出黄酮类及糖苷类代谢物。

1.5 数据统计分析

采用 SPSS 19.0 进行方差分析和 LSD 法进行多重比较。

2 结果与分析

2.1 不同施肥处理对茶树叶片光合作用的影响

如表 2 所示，胞间 CO_2 浓度 (Ci)、蒸腾速率 (E)、叶片总导度 (GTW)、CO_2 总导度 (gtc) 均以高氮

肥处理 (TF、OF) 最高，TF 处理下茶树叶片净光合速率 (A) 和气孔导度 (GSW) 显著高于 SF 处理，与 OF 处理无显著差异。这表明高氮肥促进了茶树叶片与外界环境水分和气体交换，增强了茶树光合作用。

2.2　不同施肥处理茶叶主要品质成分含量变化

茶多酚、游离氨基酸、咖啡碱、水浸出物、可溶性糖、茶氨酸是茶叶的主要品质成分。如表 3 所示，高氮肥量处理 (TF、OF) 的茶氨酸含量显著高于减氮处理 (SF)，这三者又显著高于 CK 处理，TF、OF、SF 处理茶氨酸含量较 CK 处理增加 7.22%～13.40% (P<0.05)。咖啡碱含量以 TF 处理最高，显著高于 OF、SF、CK 处理，后 3 个处理之间咖啡碱含量无显著差异。施肥显著降低了茶叶可溶性糖含量而显著增加了游离氨基酸含量，与 CK 处理相比，TF、OF、SF 处理可溶性糖含量降低了 8.17%～13.86%，游离氨基酸含量增加了 23.15%～25.50%。茶多酚含量以 CK 处理较高，高氮肥处理 TF、OF 较 CK 处理分别降低 9.35%、11.49%，氮肥减施处理 (SF) 较 CK 处理降低 6.08%，但差异不显著。水浸出物含量以高氮肥处理 (TF、OF) 较高，对照 (CK) 和减氮处理 (SF) 较低。酚氨比以 CK 处理较高，TF、OF 和 SF 处理间无显著差异。

与高氮肥处理 (TF、OF) 相比，氮肥减量 25% 并增施钾肥处理 (SF) 茶氨酸、咖啡碱含量和水浸出物含量分别降低 4.59%～5.45%、5.97% (SF 比 TF 处理)、8.91%～10.33%，而游离氨基酸、可溶性糖、茶多酚含量无显著变化。与 TF 处理相比，有机肥氮等量替代 25% 化肥氮处理 (OF) 咖啡碱含量降低 5.73%，茶多酚、茶氨酸、游离氨基酸和水浸出物含量无显著变化。

2.3　不同施肥处理茶叶中鉴定到的黄酮类及糖苷类代谢物

从图 1 可以看出，本试验检测条件下，峰形良好，分布相对均匀，数据结果可靠。

如表 4 所示，不同处理茶叶样品共检测到黄酮类代谢物 65 种、糖苷类代谢物 31 种。黄酮类代谢物包括黄酮苷 43 种、异黄酮苷 6 种、黄酮 4 种、黄

表 2　不同施肥处理茶树叶片光合生理参数

Table 2　Photosynthesis parameters of tea leaves under different fertilization treatments

处理 Treatment	A [μmol/(m²·s)]	Ci [μmol/mol]	E [mmol/(m²·s)]	GSW [mmol/(m²·s)]	GTW [mmol/(m²·s)]	gtc [mmol/(m²·s)]
CK	9.34±1.47 ab	266.3±26.7 b	5.80±1.26 b	0.23±0.24 ab	0.15±0.04 b	95.16±25.24 b
TF	10.72±2.27 a	299.4±22.1 a	8.62±0.97 a	0.27±0.04 a	0.25±0.04 a	158.63±23.07 a
OF	9.59±1.46 ab	308.5±9.15 a	8.30±0.89 a	0.27±0.04 a	0.25±0.03 a	156.23±21.95 a
SF	8.91±1.84 b	267.96±23.97 b	5.92±1.37 b	0.16±0.04 b	0.15±0.04 b	92.02±25.30 b

注：CK—不施肥处理；TF—常规化肥处理；OF—有机肥氮替代25%化肥氮处理；SF—减施25%化肥氮处理；A—净光合速率；Ci—胞间 CO_2浓度；E—蒸腾速率；GSW—气孔导度；GTW—叶片总导度；gtc—CO_2总导度。同列数据后不同字母表示处理间在5%水平差异显著。

Note: CK—No fertilizer; TF—Conventional chemical fertilizer input; OF—Replacing 25% of chemical fertilizer N with organic N; SF—Reducing 25% of chemical fertilizer N. A—Net photosynthesis rate; Ci—Intercellular carbon dioxide concentration; E—Transpiration rate; GSW— Stomatal conductance; GTW— Mesophyll conductance ; gtc— Total conductance of carbon dioxide. Values followed by different small letters in a column indicate significant difference among treatments at the 5% level.

表 3　不同施肥处理茶叶主要品质成分含量 (%)

Table 3　The contents of main quality components in tea leaves under different fertilization treatments

处理 Treatment	茶氨酸 Theanine	茶多酚 Tea polyphenols	咖啡碱 Caffeine	可溶性糖 Soluble sugar	游离氨基酸 Free amino acid	水浸出物 Water-soluble extracts	酚氨比 Polyphenols / amino acids
CK	0.97±0.03 c	17.75±0.53 a	3.97±0.03 b	4.04±0.14 a	2.98±0.33 b	46.99±1.41 b	6.01±0.75 a
TF	1.10±0.02 a	16.09±0.69 b	4.19±0.11 a	3.58±0.16 b	3.68±0.37 a	52.32±1.33 a	4.39±0.27 b
OF	1.09±0.01 a	15.71±0.33 b	3.95±0.10 b	3.48±0.08 b	3.74±0.23 a	53.15±0.51 a	4.21±0.20 b
SF	1.04±0.01 b	16.67±0.70 ab	3.94±0.05 b	3.71±0.20 b	3.67±0.27 a	47.66±1.39 b	4.55±0.15 b

注：CK—不施肥处理；TF—常规化肥处理；OF—有机肥氮替代25%化肥氮处理；SF—减施25%化肥氮处理。同列数据后不同字母表示处理间差异在5%水平显著。

Note: CK—No fertilizer; TF—Conventional chemical fertilizer input; OF—Replacing 25% of chemical fertilizer N with organic N; SF—Reducing 25% of chemical fertilizer N. Values followed by different small letters in a column indicate significant difference among treatments at the 5% level.

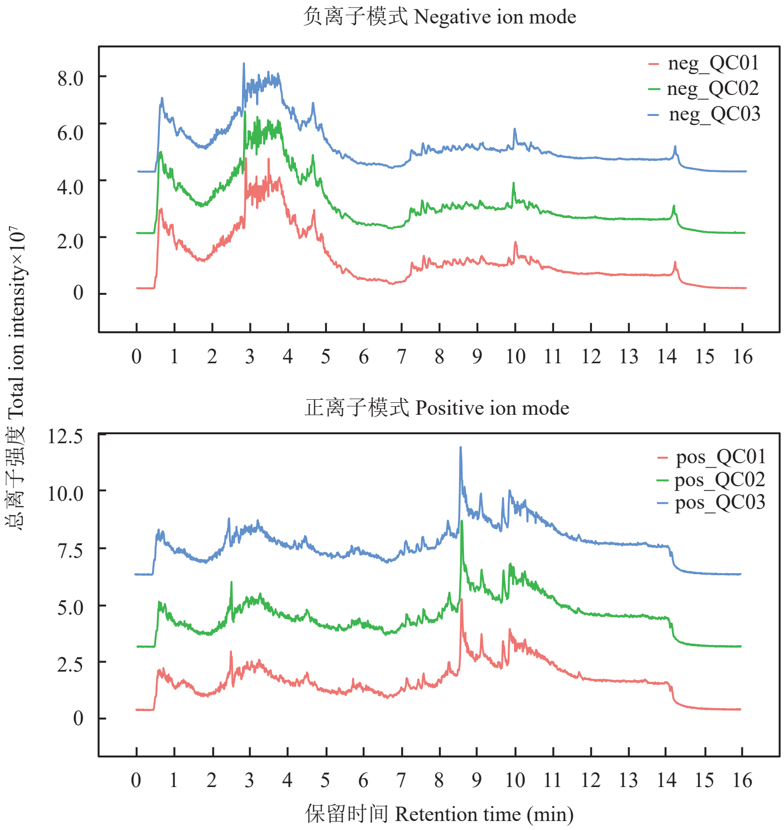

图 1　质控样本总离子流图

Fig. 1　Total ion current chromatograms of quality control sample

注：QC01 、QC02、 QC03 分别表示检测过程中采用的质控样本。

Note: QC01, QC02, QC03 mean the used quality control sample during testing, respectively.

烷醇 4 种和其它黄酮类物质 8 种。糖苷类代谢物包括萜苷 18 种、脂酰苷 7 种、甾体皂甙 5 种、香豆素糖苷 1 种。LSD 多重比较结果表明，66 种代谢物累积水平在不同施肥处理间差异显著，30 种无显著差异，说明施肥处理引起了茶叶黄酮类及糖苷类代谢物含量的改变。

2.4　减氮增钾对黄酮类及糖苷类代谢物累积水平的影响

表 5 所示，与 TF 处理相比，SF 处理显著降低了 4 种黄酮苷 (杨梅酮-3-O-洋槐糖苷、芍药素-3-O-葡萄糖苷、查耳酮樱花素、落新妇苷)，1 种黄烷醇 (表没食子儿茶素没食子酸酯)，1 种萜苷 (肉桂萜醇 C1-19-葡萄糖苷)，1 种脂酰苷 (1-辛烯-3-桑色素酊)，共 7 种代谢物累积水平，降幅 3.08%～87.21%。其中，肉桂萜醇 C1-19-葡萄糖苷降幅较大，为 87.21%。SF 显著增加了 1 种黄酮 (川陈皮素)，2 种黄酮苷 (牡荆素-4'-O-α-L-吡喃鼠李糖苷、Ranupenin 3-rutinoside) 和 1 种脂酰苷的累积水平，其中川陈皮素增幅最大，为 414.02%。

2.5　有机肥替代对黄酮类及糖苷类代谢物的影响

表 6 显示，与 TF 处理相比，OF 处理显著增加了飞燕草素-3-(6-对香豆酰基半乳糖苷)、牡荆素-4'-O-α-L-吡喃鼠李糖苷、桃皮素-3'-葡萄糖苷等 10 种黄酮苷，2 种黄酮 (杨梅酮、川陈皮素)，去氢大豆皂苷Ⅰ、珠子参苷 R2、京尼平苷酸等 6 种萜苷，苦瓜皂苷 g、葫芦素 E-2-O-葡萄糖苷、孕甾烷甙等 5 种甾体皂甙和 (S)-橙花叔醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] 等 2 种脂酰苷，共 25 种黄酮类及糖苷类代谢物累积水平。其中，川陈皮素、飞燕草素-3-(6-对香豆酰基半乳糖苷) 增幅分别达 2280.20%、1355.11%，其它 23 种代谢物累积量水平增加 10.85%～60.83%。OF 处理显著降低了 5 种黄酮苷 [杨梅酮-3-O-洋槐糖苷、芍药素-3-O-葡萄糖苷、飞燕草素-3-槐糖苷-5-葡萄糖苷、槲皮素-3-(3R-葡糖基芸香苷)、查耳酮樱花素]，2 种黄酮多聚体 (二氢黄酮、3'-没食子酰原飞燕草素 B2)，1 种异黄酮苷 (5,7-二羟基-2',6-二甲氧基异黄酮-7-鼠李糖苷)，1 种萜苷 (肉桂萜醇 C1-19-葡萄糖苷)，2 种脂酰苷 (1-辛烯-3-桑色素酊、5-大柱香波龙烷-3,9-二醇-9-[芹糖

表 4 不同施肥处理茶叶中鉴定到的黄酮类及糖苷类代谢物

Table 4 All the flavonoids and glucosides metabolites identified in tea under different fertilization treatments

编号 Number	类别 Subclass	代谢物 Metabolites	*P* 值 *P* value
1	黄酮苷 Flavonoid glucosides	槲皮素-3-O-葡萄糖苷 Quercetin-3-O-glucoside	0.5199
2		槲皮素-3-三葡萄糖甙 Quercetin-3-gentiotrioside	0.5028
3		槲皮素-3-(4-葡糖基鼠李糖苷) Quercetin-3-(4-glucosylrhamnoside)	0.5291
4		槲皮素-3-(6''-丙二酰基半乳糖苷) Quercetin-3-(6''-malonylgalactoside)	0.3126
5		3''-O-乙酰基阿福豆苷 3''-O-Acetylafzelin	0.2436
6		山奈酚-3-龙胆二糖-7-鼠李糖苷 Kaempferol-3-gentiobioside-7-rhamnoside	0.6303
7		芒花甙 Miscanthoside	0.3665
8		杞柳甙 Floribundoside	0.3403
9		3',5,6-三羟基-3,4',7,8-四甲氧基黄酮3-葡萄糖苷 3',5,6-Trihydroxy-3,4',7,8-tetramethoxyflavone 3-glucoside	0.3884
10		异牡荆素-2''-O-(6''-阿魏酰基) 葡萄糖苷 Isovitexin 2''-O-(6'''-feruloyl) glucoside	0.6195
11		芸香苷 Rutin	0.0118
12		槲皮素-3-(3-对香豆酰基葡萄糖苷) Quercetin-3-(3-p-coumaroylglucoside)	0.0343
13		槲皮素-2''-(6''-对香豆酰基葡糖基) 2''-(6''-p-Coumaroylglucosyl) quercitrin	0.0012
14		槲皮素-3-(3R-葡糖基芸香苷) Quercetin-3-(3R-glucosylrutinoside)	0.0361
15		黄芪甙 Astragalin	0.0154
16		山奈酚-3-[4''-(对香豆酰基葡糖基) 鼠李糖苷] Kaempferol-3-[4''-(p-coumaroylglucosyl) rhamnoside]	0.0026
17		山奈酚-3-[2''-(对香豆酰基葡糖基) 鼠李糖苷] Kaempferol-3-[2''-(p-coumaroylglucosyl) rhamnoside]	0.0946
18		山奈酚-3-(2'',6''-二对香豆酰基葡萄糖苷) Kaempferol-3-[2'',6''-di-(E)-p-coumarylglucoside]	0.0506
19		山奈酚-3-(2''-鼠李糖基芸香糖苷) Kaempferol-3-(2''-rhamnosylrutinoside)	0.0337
20		山奈酚-3-(6''-鼠李糖基槐糖苷) Kaempferol-3-(6''-rhamnosylsophoroside)	0.1318
21		杨梅酮-3-O-洋槐糖苷 Myricetin-3-O-robinobioside	0.0004
22		异鼠李素-3-β-昆布二糖苷 Isorhamnetin-3-β-laminaribioside	0.0001
23		异鼠李素-3-芸香糖-4'-鼠李糖苷 Isorhamnetin-3-rutinoside-4'-rhamnoside	0.0884
24		飞燕草素-3-(6-对香豆酰基半乳糖苷) Delphinidin-3-(6-p-coumaroylgalactoside)	0.0006
25		飞燕草素-3-槐糖基-5-葡萄糖苷 Delphinidin-3-sophoroside-5-glucoside	0.0063
26		花青素-3-二糖苷-5-葡萄糖苷 Cyanidin-3-diglucoside-5-glucoside	0.0389
27		芍药素-3-O-葡萄糖苷 Peonidin-3-O-glucoside	0.003
28		芹黄素-4'-[阿魏酰-(->2)-葡糖醛酸基-(1->2)-葡糖苷酸]-7-葡糖苷酸 Apigenin-4'-[feruloyl-(->2)-glucuronyl-(1->2)-glucuronide]-7-glucuronide	0.0005
29		芹黄素-7-[阿魏酰-(->2)-葡糖醛酸基-(1->2)-葡糖苷酸]-4'-葡糖苷酸 Apigenin-7-[feruloyl-(->2)-glucuronyl-(1->2)-glucuronide]-4'-glucuronide	0.0645
30		牡荆素-4'-O-α-L-吡喃鼠李糖苷 Vitexin-4'-O-α-L-rhamnopyranoside	0.0001
31		异牡荆素 Isovitexin	0.0053
32		3,3',4'-三羟基黄酮-3-O-[a-L-鼠李糖基-(1->2)[a-L-鼠李糖基-(1->6)]-β-D-吡喃葡萄糖苷] 3,3',4'-Trihydroxyflavone-3-O-[a-L-rhamnopyranosyl-(1->2)[a-L-rhamnopyranosyl-(1->6)]-β-D-glucopyranoside]	0.0751
33		查耳酮樱花素 Chalconosakuranetin	0.0009
34		锦葵色素-3-葡萄糖苷 Malvidin-3-glucoside	0.0337
35		木犀草素-7-O-[β-D-葡萄糖基-(1->2)-β-D-葡糖苷酸] Luteolin-7-O-[β-D-glucuronosyl-(1->2)-β-D-glucuronide]	0.0814

续表 4 Table 4 continued

编号 Number	类别 Subclass	代谢物 Metabolites	*P* 值 *P* value
36		6-羟基木犀草素-6-木糖苷 6-Hydroxyluteolin-6-xyloside	0.0891
37		洋李甙-4",6"-双没食子酸 Prunin-4",6"-digallic acid	0.0122
38		紫苏素 Perillanin	0.0298
39		桃皮素-3'-葡萄糖苷 Persicogenin-3'-glucoside	0.0418
40		3,5,7-三羟基-4'-甲氧基-8-异戊烯类黄酮-3-[鼠李糖基-(1->6)-半乳糖苷]-7-半乳糖苷 3,5,7-Trihydroxy-4'-methoxy-8-prenylflavone-3-[rhamnosyl-(1->6)-galactoside]-7-galactoside	0.0681
41		落新妇苷 Astilbin	0.1425
42		Ranupenin-3-rutinoside	0.1137
43		曲克芦丁 Troxerutin	0.0964
44	异黄酮 Isoflavonoids	5,7-二羟基-2',6-二甲氧基异黄酮-7-鼠李糖苷 5,7-Dihydroxy-2',6-dimethoxyisoflavone-7-rhamnoside	0.0011
45		[5,7-二羟基-3-(3-羟基-4-甲氧基苯基)-4-环氧-3,4-二氢-1-苯并吡喃醇] 丙酸 [5,7-Dihydroxy-3-(3-hydroxy-4-methoxyphenyl)-4-oxo-3,4-dihydro-2H-1-benzopyran-3-yl]methyl acetic acid	0.1961
46		西葫芦素-7-(6-丙二酰葡萄糖苷) Cicerin-7-(6-malonylglucoside)	0.1644
47		6"-丙二酰染料木甙 6"-Malonylgenistin	0.289
48		6"-O-丙二酰大豆苷 6"-O-Malonyldaidzin	0.4983
49		7-羟基-2',5,6-三甲氧基-4',5'-亚甲基二氧异黄酮-7-(2-对香豆基葡萄糖苷) 7-Hydroxy-2',5,6-trimethoxy-4',5'-methylenedioxyisoflavone-7-(2-p-coumaroylglucoside)	0.7003
50	黄烷醇 Flavanol	表儿茶素 Epicatechin	0.0186
51		表没食子儿茶素没食子酸酯 Epigallocatechin gallate	0.0610
52		表没食子儿茶素-(4β->8)-儿茶素 Epigallocatechin-(4β->8)-catechin	0.0203
53		阿萨姆霉素 C Assamicain C	0.2318
54	黄酮 Flavones	杨梅酮 Myricetin	0.096
55		川陈皮素 Nobiletin	0.0001
56		山奈酚 Kaempferol	0.7327
57		槲皮素 Quercetin	0.3093
58	其它黄酮类 Other flavonoids	二氢黄酮 Dihydromorelloflavone	0.0022
59		原花青素 C1 Procyanidin C1	0.1755
60		3'-没食子酰原飞燕草素 B2 3'-Galloylprodelphinidin B2	0.0690
61		5-羟基降山油柑碱 Bis (5-hydroxynoracronycine)	0.3770
62		表儿茶素-3-对香豆酸 Epigallocatechin-3-p-coumaroate	0.1988
63		表阿夫儿茶精-(4β->8)-表儿茶素-3,3'-双没食子酸 Epiafzelechin-(4β->8)-epicatechin-3,3'-digallate	0.8156
64		原花青素 B2 Procyanidin B2	0.3749
65		松茸苷 I Matsutakeside I	0.5367
66	萜苷 Terpene glycosides	大豆皂醇 B-3-O-β-D-葡萄糖醛酸苷 Soyasapogenol B-3-O-β-D-glucuronide	0.3016
67		28-葡萄糖基齐墩果酸-3-阿拉伯糖苷 28-Glucosyloleanolic acid-3-arabinoside	0.3150
68		大豆皂苷 Ⅰ Soyasaponin Ⅰ	0.7893
69		豌豆皂苷 Ⅱ Pisumsaponin Ⅱ	0.1925
70		10-乙酸基橄榄苦苷 10-Acetoxyoleuropein	0.1856
71		p-薄荷烷-2,8,9-三醇-2-葡萄糖苷 p-Menthane-2,8,9-triol-2-glucoside	0.7188

续表 4　Table 4 continued

编号 Number	类别 Subclass	代谢物 Metabolites	*P* 值 *P* value
72		紫苏甙 A Perilloside A	0.0016
73		反式香芹醇葡萄糖苷 (-)-trans-Carveol glucoside	0.0028
74		去氢大豆皂甙 Ⅰ Dehydrosoyasaponin Ⅰ	0.0863
75		珠子参苷 R2 Majonoside R2	0.0533
76		肉桂萜醇 A-19- 葡萄糖苷 Cinncassiol A-19-glucoside	0.0062
77		楤木皂苷 Ⅴ Araliasaponin Ⅴ	0.0141
78		牛痘苷 Vaccinoside	0.0020
79		京尼平苷酸 Geniposidic acid	0.0048
80		枸杞甙 Ⅶ Lyciumoside Ⅶ	0.0952
81		大豆皂苷Ⅱ Soyasaponin Ⅱ	0.0197
82		肉桂萜醇C1-19-葡萄糖苷 Cinncassiol C1-19-glucoside	0.0027
83		香叶酯芹糖基葡萄糖苷 Geranyl apiosyl-glucoside	0.1315
84	脂酰苷 Fatty acyl glycosides	(S)-橙花叔醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] (S)-Nerolidol-3-O-[a-L-rhamnopyranosyl-(1->2)-β-D-glucopyranoside]	0.0001
85		5-大柱香波龙烷-3,9-二醇-9-[芹糖基-(1->6)-葡萄糖苷] 5-Megastigmene-3,9-diol-9-[apiosyl-(1->6)-glucoside]	0.0040
86		3,4,5-三羟基-6-(2-甲基-3-苯丙氧基) 环氧乙烷-2-羧酸 3,4,5-trihydroxy-6-(2-methyl-3-phenylpropoxy) oxane-2-carboxylic acid	0.0913
87		棕榈酰葡糖苷酸 Palmitoyl glucuronide	0.0647
88		1-辛烯-3-桑色素酊 1-Octen-3-yl primeveroside	0.0036
89		3b,6a-二羟基-α-紫罗兰醇-9-[芹糖基-(1->6)-葡萄糖苷] 3b,6a-Dihydroxy-α-ionol-9-[apiosyl-(1->6)-glucoside]	0.7358
90		布卢门醇C-O-[鼠李糖基-(1->6)-葡萄糖苷] Blumenol C-O-[rhamnosyl-(1->6)-glucoside]	0.2408
91	甾体皂甙 Steroidal glycosides	25-乙酰氧基-3,16,20,22-四羟基-5-葫芦素-3-葡萄糖苷 25-Acetoxy-3,16,20,22-tetrahydroxy-5-cucurbiten-3-glucoside	0.0072
92		胆酸葡糖苷酸 Cholic acid glucuronide	0.0006
93		孕甾烷甙 Balagyptin	0.0058
94		葫芦素E-2-O-葡萄糖苷 Elaterinide	0.0011
95		苦瓜皂苷 g Goyaglycoside g	0.0287
96	香豆素糖苷 Coumarin glycosides	9-羟基-4-甲氧补骨脂素9-葡萄糖苷 9-Hydroxy-4-methoxypsoralen 9-glucoside	0.0012

注：*P* 值由LSD法多重比较计算得出。

Note: *P* values were calculated by least significant difference.

基-(1->6)-葡萄糖苷])，共 11 种代谢物累积量水平，降幅为 10.35%～68.89%。

2.6　不同施肥处理茶叶黄酮类及糖苷类优势代谢物鉴定

表 7 显示，根据同一代谢物在不同施肥处理茶叶中相对丰度多重比较结果，将某处理下相对丰度显著高于其它任一施肥处理的代谢物定义为该施肥处理的优势代谢物，则 CK 处理有 10 种，其中黄酮苷 4 种、黄烷醇 2 种、萜苷 2 种、脂酰苷 1 种、香豆素糖苷 1 种；OF 处理有 15 种，其中黄酮 1 种、黄酮苷 7 种、萜苷 3 种、甾体皂甙 4 种；TF 和 SF 处理未鉴定出黄酮类或糖苷类优势代谢物。OF 处理下，川陈皮素和飞燕草素-3-(6-对香豆酰基半乳糖苷) 相对丰度值较其他 3 种施肥模式的变化倍数分别达 24.89、14.52。表明，不施肥处理和有机肥替代处理均促进了黄酮类及糖苷类物质累积。

3　讨论

茶叶品质成分的形成与茶树碳、氮代谢密切相关[36]。氮是茶树营养“三要素”之一，在很大程度上

表 5 减氮增钾茶叶中累积水平显著变化的代谢物

Table 5 Metabolites with significant accumulative changes in tea leaves under reducing N and increasing K input

类别 Subclass	代谢物 Metabolites	相对丰度 Relevant abundance		变化幅度 (%) Change proportion
		SF	TF	
黄酮苷 Flavonoid glycoside	杨梅酮-3-O-洋槐糖苷 Myricetin-3-O-robinobioside	623±49.0 B	905±33.7 A	–31.13
	芍药素-3-O-葡萄糖苷 Peonidin-3-O-glucoside	38.9±6.3 B	107±23.6 A	–63.75
	查耳酮樱花素 Chalconosakuranetin	336±57.9 b	555±68.1 a	–39.56
	落新妇苷 Astilbin	259±36.4 B	318±15.6 A	–18.74
黄烷醇 Flavanol	表没食子儿茶素没食子酸酯 Epigallocatechin gallate	27300±2230 b	28200±3830 a	–3.08
萜苷 Terpene glycosides	肉桂萜醇C1-19-葡萄糖苷 Cinncassiol C1 19-glucoside	23.1±15.1 B	181±61.9 A	–87.21
脂酰苷 Fatty acyl glycosides	1-辛烯-3-桑色素酊 1-Octen-3-yl primeveroside	1040±31.7 B	1380±144.0 A	–24.58
黄酮 Flavone	川陈皮素 Nobiletin	203±3.48 A	39.4±2.04 B	414.02
黄酮苷 Flavonoid glycoside	牡荆素-4'-O-α-L-吡喃鼠李糖苷 Vitexin-4'-O-α-L-rhamnopyranoside	2640±1340 A	2380±345 B	10.84
	Ranupenin 3-rutinoside	111±15.0 a	77.0±21.3 b	44.71
脂酰苷 Fatty acyl glycosides	(S)-橙花叔醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] (S)-Nerolidol-3-O-[a-L-rhamnopyranosyl-(1->2)-β-D-glucopyranoside]	166±8.60 A	98.2±4.99 B	68.75

注：TF—常规化肥处理；SF—减施25%化肥氮处理；同行数据后不同大写字母表示1%水平差异显著，不同小写字母表示不同处理间在5%水平差异显著。

Note: TF—Conventional chemical fertilzier input; SF—Reducing 25% of chemical fertilizer N. Values followed by different capital and small letters in a row indicate difference significance between treatments at the 1% and 5% levels, respectively.

决定着茶树碳、氮代谢的平衡关系[43]，氮养分充足有利于增强茶树氮代谢[44]，提高氨基酸、咖啡碱含量；缺氮茶树氮代谢减弱，氨基酸类物质合成受阻，“碳骨架”更多被用于黄酮类及糖类物质合成[45]。本研究结果表明，不同施肥处理显著影响茶叶主要品质成分含量，在检测到的 96 种黄酮类及糖苷类代谢物中，多达 66 种代谢物的累积量在不同处理间差异显著。

与 CK 相比，施肥处理 (TF、OF、SF) 使茶叶中茶氨酸和游离氨基酸含量显著增加，茶多酚含量及酚氨比显著降低，从而有利于提升茶汤“鲜爽”度[9,46]，这与 Chen 等[44]研究结果一致。同时，高量氮肥处理 (TF) 显著增加了咖啡碱和水浸出物含量，表明适量氮肥可提升茶叶生化品质。另一方面，长期施氮会降低茶叶苯甲醇、苯乙醇、橙花叔醇等香气物质含量，不利于茶叶香气品质提升[44]，本研究中，TF、OF、SF 处理下茶叶中 (S)-橙花叔醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] 累积水平均显著低于 CK 处理。茶园不施肥会增加茶多酚[20,47]、可溶性糖含量[36]，本研究进一步表明，这可能是简单儿茶素 (表儿茶素、表没食子儿茶素)、芸香苷、槲皮苷、杨梅苷、紫苏甙 A 等主要黄酮类及糖苷类物质累积量增加所致 (表 7)。然而，不施肥处理 (CK) 茶树净光合速率未见显著下降，可能与茶树体内的氮养分转移机制[48]和低氮强烈诱导植物碳同化基因表达有关[49]，有待进一步研究。

在施氮 585 kg/hm^2 水平上氮肥减量 25% 并大幅度提高钾肥用量，茶树净光合速率、茶叶茶氨酸、咖啡碱、水浸出物含量显著低于常量化肥氮处理 (表 2、表 3)；同时，表没食子儿茶素没食子酸酯 (EGCG) 累积水平降低 3.08% (表 5)。茶氨酸和咖啡碱含量降低暗示了一定程度的氮养分缺乏。Dong 等[35]研究认为，缺氮会导致碳同化能力减弱和碳水化合物合成量降低，进而影响糖苷物质累积。本研究

表 6　有机肥替代 25% 化肥氮茶叶中累积水平显著变化的代谢物

Table 6　The metabolites with accumulation level significantly varied in tea leaves under replacing 25% of chemical fertilizer N with organic fertilizer

类别 Subclass	代谢物 Metabolites	相对丰度 Relevant abundance OF	相对丰度 Relevant abundance TF	变化幅度 (%) Change proportion
黄酮苷 Flavonoids glycoside	槲皮素-2''-(6''-对香豆酰基葡糖基) 2''-(6''-p-Coumaroylglucosyl) quercitrin	87800±3420 A	69300±2830 B	26.74
	山奈酚-3-[4''-(对香豆酰基葡糖基)鼠李糖苷] Kaempferol-3-[4''-(p-coumaroylglucosyl) rhamnoside]	71400±3940 A	57700±526 B	23.78
	牡荆素-4'-O-α-L-吡喃鼠李糖苷 Vitexin-4'-O-α-L-rhamnopyranoside	29100±596 A	23800±345 B	22.48
	异牡荆素 Isovitexin	180000±13200 A	137000±7720 B	31.23
	芹黄素-4'-[阿魏酰-(->2)-葡糖醛酸基-(1->2)-葡糖苷酸]-7-葡糖苷酸 Apigenin-4'-[feruloyl-(->2)-glucuronyl-(1->2)-glucuronide]-7-glucuronide	4180±162 A	2970±463 B	40.97
	山奈酚-3-(2'',6''-二对香豆酰基葡萄糖苷) Kaempferol-3-[2'',6''-di-(E)-p-coumarylglucoside]	2770±194 a	2350±183 b	17.80
	桃皮素-3'-葡萄糖苷 Persicogenin-3'-glucoside	1320± 100 A	985±195 B	34.10
	飞燕草素-3-(6-对香豆酰基半乳糖苷) Delphinidin-3-(6-p-coumaroylgalactoside)	405±54.8 A	27.9±10.7 B	1355.11
	Ranupenin-3-rutinoside	112±16.7 a	77.0±21.3 b	45.88
	3,3',4'-三羟基黄酮-3-O-[a-L-鼠李糖基-(1->2)[a-L-鼠李糖基-(1->6)]-β-D-吡喃葡萄糖苷] 3,3',4'-Trihydroxyflavone-3-O-[a-L-rhamnopyranosyl-(1->2)[a-L-rhamnopyranosyl-(1->6)]-β-D-glucopyranoside]	312±39.4 a	250±21.8 b	24.88
黄酮 Flavones	川陈皮素 Nobiletin	938±49.7 A	39.4±2.04 B	2280.20
	杨梅酮 Myricetin	1450±5 a	1020±116 b	41.58
萜苷 Terpene glycosides	大豆皂苷Ⅱ Soyasaponin Ⅱ	13400±1050 a	11300±581 b	18.64
	京尼平苷酸 Geniposidic acid	12300±824 A	10400±794 B	18.11
	去氢大豆皂甙Ⅰ Dehydrosoyasaponin Ⅰ	8850±440 a	7640±340 b	15.82
	珠子参苷R2 Majonoside R2	8740±315 a	7890±376 b	10.85
	肉桂萜醇A-19-葡萄糖苷 Cinncassiol A-19-glucoside	669±23.3 A	511±50.2 B	30.76
	枸杞甙Ⅶ Lyciumoside Ⅶ	583±61.3 a	458±73.7 b	27.29
甾体皂甙 Steroidal glycosides	孕甾烷甙 Balagyptin	29000±1370 A	23000±626 B	26.42
	苦瓜皂苷g Goyaglycoside g	21300±1340 a	18200±622 b	17.02
	25-乙酰氧基-3,16,20,22-四羟基-5-葫芦素-3-葡萄糖苷 25-Acetoxy-3,16,20,22-tetrahydroxy-5-cucurbiten-3-glucoside	3750±230 A	3080±220 B	21.64
	胆酸葡糖苷酸 Cholic acid glucuronide	3330±294 A	2070±318 B	60.83
	葫芦素E-2-O-葡萄糖苷 Elaterinide	415±18.7 A	280±24.1 B	48.37
脂酰苷 Fatty acyl glycosides	(S)-橙花叔醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] (S)-Nerolidol-3-O-[a-L-rhamnopyranosyl-(1->2)-β-D-glucopyranoside]	150±22.6 0A	98.2±4.99 B	52.94
	3,4,5-三羟基-6-(2-甲基-3-苯丙氧基) 环氧乙烷-2-羧酸 3,4,5-trihydroxy-6-(2-methyl-3-phenylpropoxy) oxane-2-carboxylic acid	1080±54.5 a	882±80.7 b	22.17
黄酮苷 Flavonoids glycoside	槲皮素-3-(3R-葡糖基芸香苷) Quercetin-3-(3R-glucosylrutinoside)	3970±203 b	4430±120 a	–10.35
	杨梅酮-3-O-洋槐糖苷 Myricetin-3-O-robinobioside	756±95.9 b	905±33.7 a	–16.51

续表 6 Table 6 continued

类别 Subclass	代谢物 Metabolites	相对丰度 Relevant abundance		变化幅度 (%) Change proportion
		OF	TF	
	飞燕草素-3-槐糖基-5-葡萄糖苷 Delphinidin-3-sophoroside-5-glucoside	462±39.9 B	619±10.6 A	–25.38
	查耳酮樱花素 Chalconosakuranetin	357±33.6 B	555±68.1 A	–35.70
	芍药素-3-O-葡萄糖苷 Peonidin-3-O-glucoside	61.0±24.0 B	107±23.6 A	–43.17
异黄酮苷 Isoflavonoid glycoside	5,7-二羟基-2',6-二甲氧基异黄酮-7-鼠李糖苷 5,7-Dihydroxy-2',6-dimethoxyisoflavone-7-rhamnoside	26.8±3.08 B	76.0±23.7 A	–64.76
黄酮多聚体 Polyflavonoids	3'-没食子酰原飞燕草素 B2 3'-Galloylprodelphinidin B2	127000±10800 b	175000±15100 a	–27.33
	二氢黄酮 Dihydromorelloflavone	914±48.3 B	1090±33.9 A	–16.02
萜苷 Terpene glycosides	肉桂萜醇C1-19-葡萄糖苷 Cinncassiol C1-19-glucoside	56.2±23.5 B	181±61.9 A	–68.89
脂酰苷 Fatty acylglycosides	1-辛烯-3-桑色素酊 1-Octen-3-yl primeveroside	950±104 B	1380±144 A	–31.02
	5-大柱香波龙烷-3,9-二醇-9-[芹糖基-(1->6)-葡萄糖苷] 5-Megastigmene-3,9-diol 9-[apiosyl-(1->6)-glucoside]	843±21.6 B	1140±106 A	–26.12

注：TF—常规化肥处理；OF—有机肥氮替代25%化肥氮处理。同行数据后不同大写字母表示1%水平差异显著，不同小写字母表示处理间在5%水平差异显著。

Note: TF—Conventional chemical fertilzier input; OF—Replacing 25% of chemical fertilizer N with organic N. Values followed by different capital and small letters in a row indicate difference significance between treatments at the 1% and 5% level, respectively.

中，SF 处理下，杨梅酮-3-O-洋槐糖苷、芍药素-3-O-葡萄糖苷、落新妇苷和查耳酮樱花素等 4 种黄酮苷和 1-辛烯-3-桑色素酊、肉桂萜醇 C1-19-葡萄糖苷累积水平显著降低可能与茶树净光合速率降低有关。因此，从提高茶树碳同化能力和茶叶生化品质角度，年施氮 585 kg/hm² 较 440 kg/hm² 更适合四川茶区，这一数值接近该地区茶园氮养分年平均用量 573 kg/hm²，高于我国茶园氮肥推荐用量450 kg/hm²[50]。这是由四川茶区茶园以名优绿茶原料为目标，密集采摘、蓬面多轮修剪、氮养分需求量大的生产特点所决定。

有机肥替代化肥是我国当前大力推广的施肥模式。本研究显示，施氮 585 kg/hm²，有机氮替代 25% 化肥氮处理 (OF) 与等氮量化肥处理 (TF) 相比，除咖啡碱外，茶氨酸、游离氨基酸、水浸出物、可溶性糖、茶多酚含量和酚氨比未见显著变化 (表 3)，但有 36 种黄酮类及糖苷类代谢物累积差异明显 (表 6)。OF 处理下，12 种黄酮类和 13 种糖苷类代谢物累积水平显著增加，8 种黄酮类和 3 种糖苷类代谢物累积水平显著降低。飞燕草素-3-(6-对香豆酰基半乳糖苷)、槲皮素-2''-(6''-对香豆酰基葡糖基)、山奈酚-3-[4''-(对香豆酰基葡糖基) 鼠李糖苷]、山奈酚-3-(2'',6''-二对香豆酰基葡萄糖苷) 等黄酮苷累积水平显著增加表明 OF 处理促进了茶树对香豆酸的合成，预示着茶树苯丙烷类代谢[51]途径活跃。总体来看，OF 处理促进了茶树黄酮类及糖苷类代谢物累积，有 15 种物质累积水平显著高于其它处理 (表 7)。其中，部分物质具有良好的人体保健功能，如川陈皮素[52]、芹黄素[53]、牡荆素[54]、飞燕草素[55]、槲皮素[56]等；部分糖苷物质有助于增强茶树抗逆性[57–58]，如飞燕草素及其苷能够增强植物对多种生物和非生物因素胁迫的抗性[59]；同时，糖苷是形成茶叶香气的主要前体物质，其含量增加利于提高茶叶香气品质。因此，有机肥部分替代化肥是茶园化肥减施增效的有效途径。本研究结果显示，尽管 OF 处理促进了黄酮类及糖苷类代谢物累积，但茶多酚和可溶性糖含量并未相应增加。因此，有机肥施用下，茶树体内代谢物合成转化关系尚需进一步研究。

氮养分供应影响黄酮类物质累积已在茶树和其它多种植物上得到证实[34–35]，减氮或低氮诱导黄酮类物质合成相关基因上调表达，从而促进黄酮类物质生物合成。本研究中，OF、SF 处理较 TF 处理等量减少速效氮投入，受影响的黄酮类代谢物数量分别为 20 种 (OF 较 TF)、8 种 (SF 较 TF)，表明 SF 处理对黄酮类物质的影响明显小于 OF 处理，这可能与增施钾肥提高了茶树对土壤氮的利用能力有关。同时，前人研究表明，减少氮养分促进可溶性糖和茶多酚累积，抑制氨基酸合成[36,45]，而本研究中，与常

表 7　不同施肥处理茶叶黄酮类和糖苷类优势代谢物

Table 7　Preponderant metabolites of flavonoids or glycosides in tea leaves under different fertilization treaments

类别 Subclass	代谢物 Metabolites	相对丰度 Abundance				变化倍数 Fold of change
		CK	TF	OF	SF	
黄酮苷 Flavonoid glycoside	芸香苷 Rutin	**54000±3050 a**	48500±2730 b	45800±1210 b	45000±3080 b	1.20
	槲皮素-3-(3-对香豆酰基葡萄糖苷) Quercetin-3-(3-p-coumaroylglucoside)	**67800±17800 a**	41900±4770 b	48000±7800 b	40000±2920 b	1.70
	杨梅酮-3-O-洋槐糖苷 Myricetin-3-O-robinobioside	**1040±81.1 a**	905±33.7 b	756±95.9 c	623±49.0 d	1.67
	异鼠李素-3-β-昆布二糖苷 Isorhamnetin-3-β-laminaribioside	**4430±287.0 a**	3470±93.6 bc	3760±17.7 b	3390±62.1 c	1.31
	飞燕草素-3-(6-对香豆酰基半乳糖苷) Delphinidin-3-(6-p-coumaroylgalactoside)	230.0±131 b	27.9±10.7 c	**405.0±54.8 a**	35.7±22.8 c	14.52
	芹黄素-4'-[阿魏酰-(->2)-葡糖醛酸基-(1->2)-葡糖苷酸]-7-葡糖苷酸 Apigenin-4'-[feruloyl-(->2)-glucuronyl-(1->2)-glucuronide]-7-glucuronide	2220±365 c	2970±463 b	**4180±162 a**	3010±194 b	1.89
	牡荆素-4'-O-α-L-吡喃鼠李糖苷 Vitexin-4'-O-α-L-rhamnopyranoside	21700±783 d	23800±345 c	**29100±596 a**	26400±1340 b	1.34
	异牡荆素 Isovitexin	140000±11800 b	137000±7720 b	**180000±13200 a**	147000±10900 b	1.31
	槲皮素-2''-(6''-对香豆酰基葡糖基) 2''-(6''-p-Coumaroylglucosyl) quercitrin	68400±5980 b	69300±2830 b	**87800±3420 a**	75200±2910 b	1.28
	山奈酚-3-[4''-(对香豆酰基葡糖基)鼠李糖苷] Kaempferol-3-[4''-(p-coumaroylglucosyl) rhamnoside]	57700±5110 b	57700±526 b	**71400±3940 a**	61000±1140 b	1.24
	3,3',4'-三羟基黄酮-3-O-[a-L-鼠李糖基-(1->2)[a-L-鼠李糖基-(1->6)]-β-D-吡喃葡萄糖苷] 3,3',4'-Trihydroxyflavone-3-O-[a-L-rhamnopyranosyl-(1->2) [a-L-rhamnopyranosyl-(1->6)]-β-D-glucopyranoside]	237±44.80 b	250±21.80 b	**312±39.40 a**	248±9.26 b	1.32
黄酮 Flavone	川陈皮素 Nobiletin	37.7±2.46 c	39.4±2.04 c	**938±49.70 a**	203±3.48 b	24.89
黄烷醇 Flavanol	表没食子儿茶素-(4β->8)-儿茶素 Epigallocatechin-(4β->8)-catechin	**73800±16600 a**	46200±4040 b	54000±7820 b	45100±2930 b	1.63
	表儿茶素 Epicatechin	**19000±1790 a**	16600±238 b	15900±435 b	16200±800 b	1.20

续表 7 Table 7 continued

类别 Subclass	代谢物 Metabolites	相对丰度 Abundance				变化倍数 Fold of change
		CK	TF	OF	SF	
萜苷 Terpene glycosides	紫苏甙 A Perilloside A	**15000±2540 a**	6230±544 b	9380±1880 b	7000±1860 b	2.41
	反式香芹醇葡萄糖苷 (-)-trans-Carveol glucoside	**718±115 a**	287±32.4 b	471±122 b	320±104 b	2.50
	去氢大豆皂甙 I Dehydrosoyasaponin I	7620±860 b	7640±340 b	**8850±440 a**	7710±559 b	1.16
	珠子参苷 R2 Majonoside R2	7850±245 b	7890±376 b	**8740±315 a**	7530±717 b	1.16
	肉桂萜醇 A-19-葡萄糖苷 Cinncassiol A-19-glucoside	458±77.2 b	511±50.2 b	**669±23.3 a**	540±41.4 b	1.50
甾体皂甙 Steroidal glycosides	25-乙酰氧基-3,16,20,22-四羟基-5-葫芦素-11-one 3-葡萄糖苷 25-Acetoxy-3,16,20,22-tetrahydroxy-5-cucurbiten-11-one-3-glucoside	3140±63 b	3080±220 b	**3750±230 a**	3090±204 b	1.22
	胆酸葡糖苷酸 Cholic acid glucuronide	2200±169.0 c	2070±318.0 c	**3330±294.0 a**	2810±57.7 b	1.61
	孕甾烷甙 Balagyptin	25400±2300 b	23000±626 b	**29000±1370 a**	24900±905 b	1.26
	葫芦素E-2-O-葡萄糖苷 Elaterinide	311±35.6 b	280±24.1 b	**415±18.7 a**	251±42.6 b	1.65
脂酰苷 Fatty acyl glycosides	(S)-橙花叔醇-3-O-[a-L-鼠李糖吡喃醇-(1->2)-β-D-吡喃葡萄糖苷] (S)-Nerolidol-3-O-[a-L-rhamnopyranosyl-(1->2)-β-D-glucopyranoside]	**198±9.15 a**	98.2±4.99 c	150±22.60 b	166±8.60 b	2.01
香豆素糖苷 Coumarin glycoside	9-羟基-4-甲氧补骨脂素9-葡萄糖苷 9-Hydroxy-4-methoxypsoralen 9-glucoside	**2070±184 .0a**	1450±161.0 b	1380±90.6 b	1590±95.7 b	1.50

注：CK—不施肥处理；TF—常规化肥处理；OF—有机肥氮替代25%化肥氮处理；SF—减施25%化肥氮处理。变化倍数为同一代谢物在不同施肥处理间最大相对丰度值与最小相对丰度值之比。同行数据后不同小写字母表示处理间在5%水平差异显著。

Note: CK—No fertilizer; TF—Conventional chemical fertilizer input; OF—Replacing 25% of chemical fertilizer N with organic N; SF—Reducing 25% of chemical fertilizer N. Change fold was the ratio of maximum to minimum abundance of a same metabolite among the four treatments. Values followed by different small letters in a row indicate difference significance among different treatments at the 5% level.

规化肥处理相比，氮肥减量 25% (SF) 未引起三者含量及酚氨比的显著变化，进一步证实了增施钾肥对茶树吸收利用土壤氮的促进作用。从有差异的黄酮类及糖苷类物质数量看，OF 处理较 TF 处理有 36 种，SF 处理较 TF 处理有 11 种，说明 OF 处理较 SF 处理对茶树次级代谢产物的影响更强烈，这与有机肥养分种类丰富和改善土壤理化性状能力强有关[21,60]。另外，本研究发现，与 TF 处理相比，杨梅酮-3-O-洋槐糖苷、芍药素-3-O-葡萄糖苷、牡荆素-4'-O-α-L-吡喃鼠李糖苷等 9 种物质累积水平在 OF、SF 处理表现出相似增减趋势，推测茶叶中糖苷物质对氮养分的响应与黄酮类物质相似。

4 结论

茶园施肥显著影响着茶叶品质成分含量及其组成。与常规化肥处理相比，以有机肥替代 25% 化肥氮降低了茶叶咖啡碱含量，提高了川陈皮素、飞燕草素、槲皮素、芹黄素、山奈酚、牡荆素等多种黄酮类代谢物和去氢大豆皂甙 I 、珠子参苷 R2、孕甾烷甙等多种糖苷组分累积水平。减施 25% 氮肥降低了茶氨酸、咖啡碱、水浸出物含量和糖苷类代谢物(如杨梅酮-3-O-洋槐糖苷、芍药素-3-O-葡萄糖苷、肉桂萜醇 C1-19-葡萄糖苷等) 累积水平，对茶多酚、可溶性糖、游离氨基酸含量和酚氨比影响较小。

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