

Proton release by tea plant (*Camellia sinensis* L.) roots as affected by nutrient solution concentration and pH

Q. Wan^{1,2}, R.K. Xu¹, X.H. Li²

¹State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, P.R. China

²Tea Science Research Institute, Nanjing Agriculture University, Nanjing, P.R. China

ABSTRACT

Solution culture experiments were conducted and the protons released were measured with an automatic titration system to determine the main factors affecting proton release by tea roots. Results indicated that the higher were the cation concentrations, the more protons were released from the roots, suggesting that tea roots took up a large amount of cations during growth, and then released protons to maintain charge balance of the plant body. The amount of protons released from tea roots at controlled pH was much higher than that in the treatments with uncontrolled pH. Stepwise multiple linear regression analysis showed that both NH_4^+ and Al(III) played distinct roles in proton release by tea plant roots. The uptake of Al(III) and NH_4^+ and subsequent release of protons may be an important mechanism for soil acidification in tea gardens.

Keywords: acidification; aluminum; ammonium; charge balance; tea gardens

Tea (*Camellia sinensis* L.) is a major commercial crop in many countries in tropical and subtropical regions, including China. Plantations of tea accelerate soil acidification, and acidification degree of tea garden soils increased with cultivation time (Abe et al. 2006, Oh et al. 2006, Wang et al. 2010). An acidic environment is favorable for tea plant growth, but if soil pH is too low and levels of aluminum (Al) are high there can be negative impacts on tea plants (Fung et al. 2008); a range of suitable pH for tea plants is 4.5–6.0. Ammonium (NH_4^+)-N fertilizers applied to increase tea yields accelerate soil acidification through the nitrification of NH_4^+ (Ruan et al. 2004, Oh et al. 2006). Tea plants are known to be typical Al accumulators and can take up large amounts of Al, most of which accumulates in leaves. Biogeochemical cycling of Al in tea litter is also considered a cause of soil acidification in tea plantations, which may occur at an early stage of tea cultivation, because incorporation of tea litter increased pH of the strongly acidified soils from tea gardens (Ruan et al. 2004).

Proton release by the roots of several crops has been reported. Taylor and Bloom (1998) found that the presence of NH_4^+ alone led to a dramatic increase in net H^+ extrusion in root apex regions. P deficiency enhanced excess cation uptake and concomitant proton release by white lupin and tomato (Imas et al. 1997, Shen et al. 2004). It is well known that the cultivation of leguminous plants leads to soil acidification. The release of protons by roots of leguminous plants, due to the unbalanced uptake of more cations than anions, is the main mechanism (Bolan et al. 1991, McLay et al. 1997). Similarly, tea plants take up large amounts of Al(III) and NH_4^+ -N from soils during growth, and it could be expected that the uptake of excess cations by tea plants also leads to the release of protons by their roots and thus accelerates soil acidification. However, little information is available on the protons release by tea plant roots and their potential impact on soil acidification. The objective of the present study was to investigate the effects of the main cations on quantity of protons released from tea roots.

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MATERIAL AND METHODS

Culture experiments. Two-year-old rooted cuttings of tea cv. Longjing-changye from a tea plantation in Nanjing, China were used. The roots, shoots and leaves of the tea plants were washed with tap water three times, followed by distilled water a further three times. Uniform tea plants were selected and mounted in plastic lids on the top of 1-L buckets, with one plant per bucket. Firstly, tea plants were immersed in tap water for 3 days and then exposed to one-third-concentration nutrient solution for one week, half-concentration nutrient solution for one week, three-quarter-concentration nutrient solution for one week and full nutrient solution for 2–3 weeks. The nutrient solution in buckets contained mass elements: NH_4^+ -N, Ca, K, Al, Mg of 750, 395, 350, 250, 210 μmol and trace elements: B, Fe as Fe-EDTA, Zn, Mn, Mo and Cu of 3.33, 2.1, 0.51, 0.50, 0.17 and 0.13 μmol – this nutrient solution was termed ‘full nutrient solution’. The concentrations of SO_4^{2-} , Cl^- , PO_4^{3-} and NO_3^- in full nutrient solution are 1111, 540, 50 and 250 μmol . Then a series of nutrient solutions were prepared with concentrations of NH_4^+ , K, Ca, Mg, Al, H_2PO_4^- , SO_4^{2-} , Cl^- and NO_3^- of 1/4, 1/2, 3/4, 1.0, 1.5 and 2.0 fold of those in the full nutrient solution, while the concentrations of B, Mn, Zn, Cu, Mo and Fe remained the same for all nutrient solutions. The pH of these nutrient solutions were adjusted to 5.0 with H_2SO_4 or NaOH.

When many new white roots had grown out, the tea plants were moved from the full nutrient solution to the different treatment solutions. After 7 days of cultivation, the concentrations of Al(III), Ca^{2+} , Mg^{2+} , K^+ , NH_4^+ and H^+ in the cultured solutions and the solution pH were measured. The amounts of Al(III), Ca^{2+} , Mg^{2+} , K^+ and NH_4^+ taken up by tea plants were calculated from the difference in the concentrations of these elements and the difference in nutrient solution volume before and after tea plant growth. The culture experiments were conducted in a greenhouse, with three replicates per treatment.

Effect of initial solution pH on proton release by tea plant roots. The full nutrient solution was prepared with the method mentioned above and then the solution pH was adjusted to 4.0, 4.5 or 5.0 with H_2SO_4 or NaOH. Then the same methods as mentioned above were used to investigate the initial solution pH on proton release by tea plant roots.

The release of protons by tea plant roots under controlled pH. The full nutrient solution was

prepared and then the solution pH was adjusted to 4.0, 4.5 or 5.0 with H_2SO_4 and NaOH. One tea plant was placed in one of these nutrient solutions and the solution pH maintained constant using an automatic titration system (TIM 854, Radiometer, Rue d'Alsace, France) through addition of NaOH to neutralize the protons released from roots during plant growth. The culture experiment lasted for 12 h. At the same time, the culture experiment with uncontrolled solution pH was conducted for 12 h. After 12 h of cultivation, the protons released from tea plant roots and the concentrations of Al(III), Ca^{2+} , Mg^{2+} , K^+ and NH_4^+ in the cultured solutions were determined.

Analyses of culture solutions after culture experiments. The pH of the nutrient solution was measured using an Orion 720 pH meter (Boston, USA) with a combination electrode. The protons in the solutions were titrated with 0.01 mol/L NaOH using the TIM 854 (Rue d'Alsace, France). For controlled pH experiments, the solution pH was maintained using the automatic titration system through addition of NaOH to neutralize the protons being released from tea plant roots during cultivation. The proton release was deduced from the amount of NaOH delivered by the automated dispenser. Soluble Al was determined by the 8-hydroxyquinoline method at pH 8.3, using butyl acetate as an extractant (Li et al. 2005). Ca^{2+} and Mg^{2+} in culture solution were determined by atomic absorption spectrophotometry, and K^+ by flame photometry. NO_3^- and NH_4^+ in culture solution were determined by the continuous flow analytical system (Skalar San++, Tinstraat, The Netherlands). Phosphate in culture solutions was determined by the ascorbic acid-molybdate blue method, and Cl^- by potentiometric titration which was titrated with 0.01 mol/L AgNO_3 using the TIM 854. Sulfate in culture solutions was determined by ion chromatography (Dionex DX-500, Sunnyvale, USA).

Statistical analysis. Analysis of stepwise multiple linear regression was performed using the software SPSS 17.0 for Windows (Chicago, USA). The analysis of variance (ANOVA) was used to establish the relationship between proton release and uptake of cations by tea plants. Least significant difference (LSD) was used to compare treatment means.

RESULTS AND DISCUSSION

Proton release as affected by different amounts of the main cations. At the end of 7th day of culture, the proton release by tea roots increased with

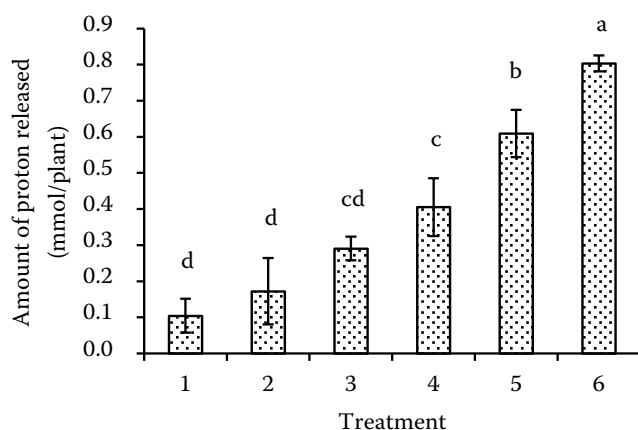


Figure 1. Amount of protons released from tea plant roots after 7 day of growth of the plants in different nutrient solutions. Treatments 1–6 represent 1/4, 1/2, 3/4, 1.0, 1.5 and 2.0 fold of concentrations of NH_4^+ , Al(III) , Ca^{2+} , Mg^{2+} , and K^+ in the nutrient solution. Standard errors (\pm) of each point are shown with a vertical bar. Means followed by the same letter above data columns are not significantly different ($P < 0.05$)

increased concentrations of Al(III) , NH_4^+ , Ca^{2+} , Mg^{2+} and K^+ (Figure 1). This suggested that the higher concentrations of the main cations induced higher release of protons by the roots. Statistical analysis indicated significant ($P < 0.05$) differences in proton release among the treatments of 1.0, 1.5 and 2.0 fold-nutrient solutions, while the difference in pH changes among these treatments was not significant. The greater buffering of the higher Al concentration in these media to acidity led to less change in medium pH for these treatments. Therefore, the amounts of protons released from roots could reflect the impact of tea plant growth on acidification more than change of the medium pH.

The amount of Al(III) , NH_4^+ , Ca^{2+} , Mg^{2+} and K^+ took up by tea plants during culture experiments

(Table 1) indicated that NH_4^+ uptake by tea plants significantly ($P < 0.05$) increased with increased concentrations of the cation in nutrient solution. Similar trends were observed for the uptake of Ca^{2+} by tea plants, but the differences in Ca^{2+} uptake between the treatments of 1/4 and 1/2 fold, and between 1/2 and 3/4 fold-nutrient solutions were not significant. The uptake of Al(III) by tea plants was also increased with the increased cation concentrations in the nutrient solutions; the increase was significant ($P < 0.05$) from 1.0 to 1.5 fold, and then to 2.0 fold-nutrient solution. The uptake of Mg^{2+} and K^+ by tea plants did not significantly increase with increased concentrations of these cations. The uptake of inorganic anions by tea plants was also presented in Table 1. The difference between the amount of total cations and

Table 1. Uptake of main cations and anions by tea plants calculated from the difference in concentrations of these ions and the difference in nutrient solution volume after cultured in nutrient solutions with different initial concentrations of nutrient ions for 7 days

Nutrient solution	Al(III)	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	H_2PO_4^-	SO_4^{2-}	Cl^-	NO_3^-	Difference
(mmol ₍₊₎ /plant)										
1/4 times	0.36 $\pm 0.06^d$	0.13 $\pm 0.00^f$	0.08 $\pm 0.01^b$	0.16 $\pm 0.02^e$	0.10 $\pm 0.02^b$	0.01 $\pm 0.00^f$	0.16 $\pm 0.02^c$	0.11 $\pm 0.01^b$	0.07 $\pm 0.00^b$	0.48
1/2 times	0.62 $\pm 0.03^{cd}$	0.28 $\pm 0.00^e$	0.08 $\pm 0.01^b$	0.22 $\pm 0.04^{de}$	0.20 $\pm 0.02^{ab}$	0.03 $\pm 0.00^e$	0.22 $\pm 0.04^c$	0.12 $\pm 0.01^{ab}$	0.13 $\pm 0.00^b$	0.90
3/4 times	0.72 $\pm 0.06^{cd}$	0.43 $\pm 0.00^d$	0.11 $\pm 0.01^{ab}$	0.36 $\pm 0.02^d$	0.16 $\pm 0.04^b$	0.04 $\pm 0.00^d$	0.32 $\pm 0.08^{bc}$	0.15 $\pm 0.02^a$	0.17 $\pm 0.02^b$	1.10
1 time	0.87 $\pm 0.09^c$	0.58 $\pm 0.00^c$	0.14 $\pm 0.02^a$	0.54 $\pm 0.06^c$	0.22 $\pm 0.02^a$	0.05 $\pm 0.00^c$	0.94 $\pm 0.16^a$	0.04 $\pm 0.00^c$	0.25 $\pm 0.00^{ab}$	1.07
1.5 times	1.26 $\pm 0.12^b$	0.74 $\pm 0.07^b$	0.06 $\pm 0.01^b$	0.86 $\pm 0.12^b$	0.26 $\pm 0.02^a$	0.07 $\pm 0.00^b$	0.54 $\pm 0.24^b$	0.04 $\pm 0.02^c$	0.29 $\pm 0.06^{ab}$	2.24
2 times	1.80 $\pm 0.09^a$	1.09 $\pm 0.08^a$	0.10 $\pm 0.02^{ab}$	1.40 $\pm 0.02^a$	0.26 $\pm 0.02^a$	0.09 $\pm 0.00^a$	0.52 $\pm 0.04^b$	0.03 $\pm 0.01^c$	0.38 $\pm 0.12^a$	2.36

Difference – total concentrations of cations – total concentrations of anions. Values are means \pm SE of 3 replicates. Means followed by the same letter within a row are not significantly different ($P < 0.05$)

Table 2. Proton release and uptake of the five cations by tea plants cultured in nutrient solutions with different initial pH after 7 days

Initial pH	Final pH	Release of H ⁺ (mmol/plant)	Uptake of cations by tea trees (mmol/plant)				
			Al(III)	NH ₄ ⁺	K ⁺	Ca ²⁺	Mg ²⁺
5.0	3.69 ± 0.07	0.51 ± 0.06 ^a	0.18 ± 0.02 ^b	0.74 ± 0.01 ^a	0.07 ± 0.01 ^a	0.23 ± 0.04 ^a	0.07 ± 0.01 ^a
4.5	3.38 ± 0.04	0.53 ± 0.05 ^a	0.24 ± 0.00 ^a	0.72 ± 0.01 ^a	0.07 ± 0.01 ^a	0.22 ± 0.04 ^a	0.06 ± 0.00 ^a
4.0	4.16 ± 0.01	-0.01 ± 0.00 ^b	0.06 ± 0.00 ^c	0.73 ± 0.01 ^a	0.09 ± 0.02 ^a	0.23 ± 0.00 ^a	0.07 ± 0.00 ^a

Values are means ± SE of 3 replicates. Means followed by the same letter within a row are not significantly different ($P < 0.05$)

total anions took up by tea plants was calculated (Table 1). Results indicated that the uptake of total cations by tea plants was much greater than that of total anions.

Contribution of different cations to proton release for the treatments of different-fold nutrient solutions. Stepwise multiple linear regression was used to compare the relative contribution of the five cations to proton release. Proton release by tea plant roots was the dependent variable and uptake by tea plants of Al(III), NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ were the independent variables. The five independent variables were added to the linear regression in a stepwise method, and the independent variables which were not significantly related to proton release were removed from the regression equation. The analysis of variance (ANOVA) for the model ($F = 227.446$, $P < 0.001$) indicated a linear relationship between proton release by tea plant roots and NH₄⁺ uptake, suggesting that NH₄⁺ uptake could be used as an explanatory variable to explain the variation of proton release by tea plant roots. The regression equation was:

$$Y = 0.776X \text{ in mmol by plant} \quad (1)$$

where: Y – proton release by tea plant roots; X – NH₄⁺ uptake.

Effect of initial medium pH on proton release.

Proton release was not detected at pH 4.0, while tea plants released protons into nutrient solutions under both pH 4.5 and 5.0 conditions. The amount of protons released at pH 4.5 was slightly greater than that at pH 5.0, but the difference between the two treatments is not significant (Table 2). The uptake of NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ by tea plants did not change significantly with change of medium pH (Table 2), while the uptake of Al by tea plants increased significantly with higher initial pH ($P < 0.05$).

Controlled pH (constant pH) experiments simulated the release of protons by tea plant roots under field conditions. The dynamics of proton release indicated that the amount of protons released increased with culture time (Figure 2). The release of protons also significantly increased when initial medium pH increased from 4.0 to 4.5, similarly to observations under uncontrolled pH (non-constant pH) conditions (Table 2). These results support the interpretation that the relatively higher pH conditions resulted in more protons being released from tea plant roots.

The amount of protons released from tea plant roots at controlled pH was much higher than that under uncontrolled pH conditions when the other

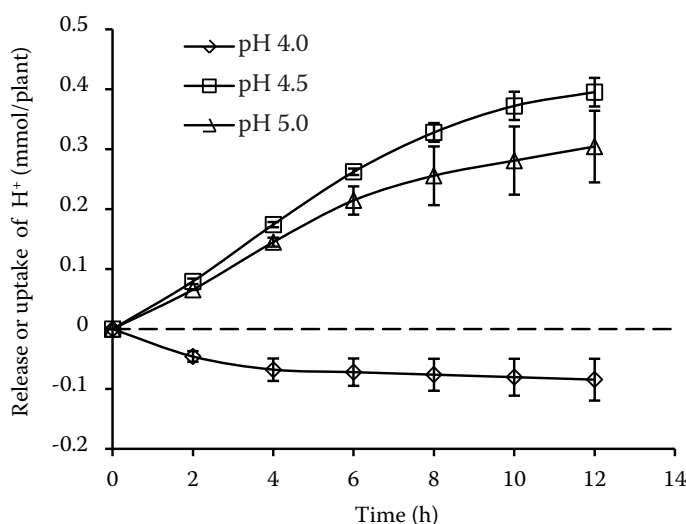


Figure 2. Dynamics of protons released from tea plant roots under controlled pH (constant pH) conditions, with different initial pH. Standard errors (±) of each point are shown with a vertical bar. Negative value represents the uptake of H⁺

Table 3. Comparison of the five cations uptake between constant pH and non-constant pH conditions for tea plants cultured in different initial pH nutrient solution after 12 h

Cation	pH 5.0		pH 4.5		pH 4.0	
	constant pH	non-constant pH	constant pH	non-constant pH	constant pH	non-constant pH
(mmol/plant)						
Al ³⁺	0.13 ± 0.02	0.09 ± 0.01	0.17 ± 0.03	0.14 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
NH ₄ ⁺	0.29 ± 0.10	0.20 ± 0.01	0.28 ± 0.06	0.19 ± 0.02	0.12 ± 0.05	0.14 ± 0.03
K ⁺	0.07 ± 0.00	0.05 ± 0.03	0.06 ± 0.03	0.03 ± 0.01	0.08 ± 0.01	0.05 ± 0.02
Ca ²⁺	0.10 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.06 ± 0.00	0.06 ± 0.02	0.09 ± 0.01
Mg ²⁺	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00

Values are means ± SE of 3 replicates

experimental conditions were the same, especially for higher initial pH (Figure 3). The amounts of the five cations taken up by tea plants during 12 h of cultivation (Table 3) showed that the uptake of Al³⁺ and NH₄⁺ by tea plants under controlled pH conditions was significantly higher than that for uncontrolled pH ($P < 0.05$) in the treatments of initial pH 4.5 and 5.0. However, the difference between Ca²⁺ and Mg²⁺ took up by tea plants between controlled and uncontrolled pH was not significant. The amount of K⁺ took up by tea plants both at controlled and uncontrolled pH was much lower than Al³⁺ and NH₄⁺ took up, thus the uptake of K⁺ contributed little to the protons released from roots.

Soil pH is an important factor influencing the uptake of Al(III) by tea plants and the subsequent release of protons by the tea plant roots. The results from this study indicated that tea plants took up more Al(III), leading to an increase in the amount of protons being released from their roots at pH 4.5 compared to pH 5.0 because soluble Al(III) and free Al³⁺ increased with decreasing pH, while the too low pH and high concentration of Al(III) can

cause toxicity of H⁺ and Al³⁺ to tea plants. This may be the reason for no protons released from tea plant roots at pH 4.0.

Contribution of different cations to proton release at different pH and controlled pH. Stepwise multiple linear regression with experimental data (Figure 3, Tables 2–3) was used to compare the relative contribution of the five cations to proton release at different pH; with proton release the dependent and uptakes of Al(III), NH₄⁺, K⁺, Ca²⁺ and Mg²⁺ the independent variables. The ANOVA for the model ($F = 87.096$, $P < 0.001$) indicated a linear relationship between proton release by roots and Al(III) uptake. Partial regression of the independent variable in the model using ANOVA indicated significant probabilities of the constant and uptake of Al(III) were 0 and 0.046, respectively, thus suggesting that the following regression equation could be used to explain the variation of proton release by tea plant roots:

$$Y = -0.1 + 2.856X \quad (2)$$

Where: Y – proton release by tea plant roots (mmol/plant);
 X – the uptake of Al(III).

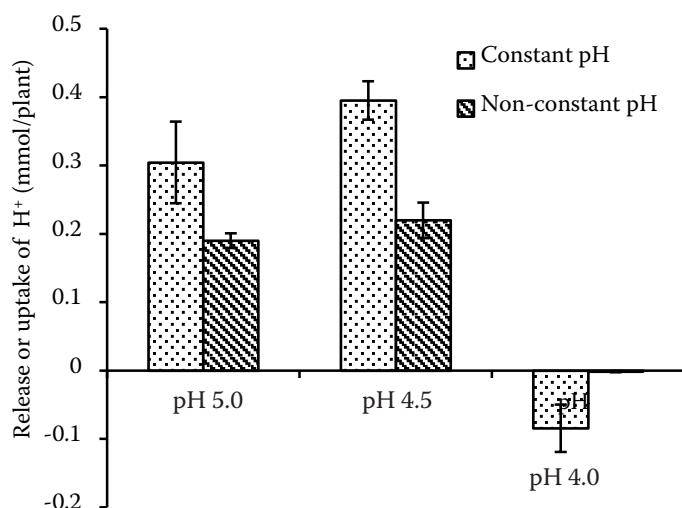


Figure 3. Comparison of protons released from tea plant roots for treatments of constant and non-constant pH with different initial pH. Standard errors (±) of each point are shown with a vertical bar. Negative value represents the uptake of H⁺

The two regression equations [equations (1) and (2)] showed significant correlations between proton release by tea plant roots and the uptake of Al(III) and NH_4^+ . Tea prefers NH_4^+ as its inorganic nitrogen source. Tea plants are also known to be typical Al accumulators and can take up large amounts of Al. Therefore, the uptake of Al(III) and NH_4^+ by tea plants induced proton release by tea roots and thus led to medium acidification.

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Corresponding authors:

Prof. Ren-Kou Xu, Chinese Academy of Sciences, Institute of Soil Science, P.O. Box 821, Nanjing, P.R.China
phone: + 86 25 8688 1183, fax: + 86 25 8688 1000, e-mail: rkxu@issas.ac.cn

Prof. Xing-Hui Li, Nanjing Agriculture University, Tea Science Research Institute, Nanjing 210095, P.R. China
phone: +86 25 84395182, fax: +86 25 84395182, e-mail: lxh@njau.edu.cn
