Effects of Soil Type and Soil Treatment on Solubilization of 13 Elements in the Root Zone and their Absorption by Blueberry Bushes

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In blueberry culture, sulfur is applied to enhance soil acidification, and fertilizer is applied to increase growth and yield. We investigated the effects of soil management on the solubilization of 13 elements in the root zone and their relationships with absorption by blueberry bushes. In a 2-year pot experiment, four-year-old rabbiteye blueberries ‘Onslow’ were grown in one of three soils (an Andosol, a Cambisol, or a Fluvisol), with or without soil treatment (no treatment, acidification, fertilization, or acidification plus fertilization). The soil solution was collected eight times during the experiment. Fruit, leaves, branches, stems, and roots were sampled during and at the end of the experiment. The concentrations of 13 elements (N, Na, Mg, Al, P, K, Ca, Mn, Fe, Cu, Zn, Rb, and Cs) in the samples were analyzed. Soil solution pH was also measured. In all soils, the soil solution pH was decreased to 3.7–4.3 by acidification and also to 4.5–6.1 by fertilization. Acidification tended to increase the average concentrations of Al (77–1421 fold), Zn (18–414 fold), and Fe (1.2–204 fold) in the soil solution, whereas fertilization tended to increase the average concentrations of NH4+ (33–205 fold), Cs (3.0–9.9 fold), and NO3− (2.1–8.4 fold). The acidification plus fertilization further increased the concentrations of these elements in the soil solution except for Fe. On the other hand, the concentrations of Na, P, Fe, and Cu in the soil solution were influenced by the soil type and were not changed by any soil treatments in a particular soil. Across all soil types and treatments, the average concentrations of N, P, K, Mn, Cu, and Zn in the soil solution were significantly correlated with the content of the corresponding element in the blueberry bushes. For these elements, nondestructive sampling and analyses of soil solution in the root zone can be effective as a real-time soil test. The content of seven other elements (Na, Mg, Al, Ca, Fe, Rb, and Cs) in the bushes did not reflect the soil solution concentrations partly due to the lower requirement than their supply from the soil.

Key Words: acidification, fertilization, rabbiteye blueberry, root-zone soil solution, soil type.
In relation to blueberry culture, however, little is known about the effects of unique soil management on the concentrations of various elements in the soil solution and on their absorption by blueberry bushes. Such comprehensive information would be valuable not only for the rational soil management of blueberries, but also for a better understanding of the nutrient acquisition by blueberry bushes grown in various soil conditions. In this study, therefore, we evaluated the dynamics of 13 absorption by blueberry bushes grown in different soil conditions. In this study, therefore, we evaluated the dynamics of 13 absorption by blueberry bushes grown in different soil conditions.

Materials and Methods

Pot experiment

The pot experiment was composed of three types of soils (an Andosol, a Cambisol, or a Fluvisol) combined with four soil treatments (no treatment, acidification, fertilization, or acidification plus fertilization). Each treatment was done in triplicate, and a total of 36 pots were prepared.

The three soils were collected from the surface layer (0–20 cm) of experimental orchards, Tsukuba, Kasumigaura, or Takatsuki in Japan (Table 1), air-dried, and passed through a 2-mm sieve. As shown in Table 1, Tsukuba soil was highest in total C, and Kasumigaura soil was lowest in pH and total C. Takatsuki soil contained higher amounts of P, Fe, Cu, and Zn in water soluble form than other two soils. Four-year-old rabbit-eye blueberries ‘Onslow’ were planted in all pots. The plants were purchased from a nursery company in Japan and then pruned conventionally on 18–19 February 2014. For the acidification treatment, we thoroughly mixed 3 kg of soil with sulfur powder at 5 g·kg−1, which was assumed to give a final soil pH of 3 to 4 from preliminary incubation results. For the fertilization treatment, we thoroughly mixed 3 kg of soil with 2.29 g NH4Cl at 0.2 g N·kg−1 and 1.14 g KCl at 0.2 g K·kg−1. The water content was adjusted to 60% of the maximum water-holding capacity with tap water. In order to maintain aerated soil conditions for blueberry roots, a 7 L plastic pot with an open bottom (210 mm height, 240 mm diameter) was used. To collect soil solution samples nondestructively, two porous cup samplers (DIK-8393; Daiki Rika Kogyo, Saitama, Japan) were placed in the plastic pot at 5–6 cm above the bottom, supported by adding soil beneath them (Fig. 1, left), and then covered with part of the remainder of the soil. The blueberry bushes were then planted by adding the rest of the soil to the pots (Fig. 1, middle).

The date when the bushes were transplanted in the pots was referred to as day 0 of the experiment. The experiment was conducted for a total period of 548 days in a glass room in Tsukuba, Japan, from 18 March 2014 to 17 September 2015. On 30 March 2015 in the second year of cultivation, the pots with the fertilization treatment were treated with additional fertilization at the same application rates (NH4Cl at 0.2 g N·kg−1, and KCl at 0.2 g K·kg−1) by adding the dissolved solution to the soil surface of the pot. The average temperature in the glass room was 16.6°C during the period from 30 October 2014 to 28 September 2015. Pots were watered daily.

| Soil origin | WRB | pH (H2O) | EC (mS·m−1) | Total C (g·kg−1) | Total N (g·kg−1) | Sand (%) | Silt (%) | Clay (%) | Soil texture |
|-------------|-----|----------|-------------|-----------------|-----------------|----------|----------|----------|-------------|
| Tsukuba     | Andosol | 6.4 | 8.1 | 40 | 3.2 | 47 | 34 | 19 | clay loam |
| Kasumigaura | Cambisol | 5.7 | 6.2 | 24 | 2.2 | 66 | 22 | 12 | sandy loam |
| Takatsuki   | Fluvisol | 6.7 | 10 | 28 | 3.0 | 61 | 22 | 17 | clay loam |

| Soil origin | Water soluble concentrationa |
|-------------|-----------------------------|
|              | NH4+ (mg L−1) | NO3− (mg L−1) | Na (mg L−1) | Mg (mg L−1) | Al (mg L−1) | P (mg L−1) | K (mg L−1) | Ca (mg L−1) | Mn (mg L−1) | Fe (mg L−1) | Cu (mg L−1) | Zn (mg L−1) | Rb (μg L−1) | Cs (μg L−1) |
| Tsukuba     | 0.35 | 0.22 | 0.92 | 10.7 | 3.03 | 0.09 | 44.9 | 40.7 | 0.09 | 0.65 | 0.00 | 0.02 | 21.1 | 0.08 |
| Kasumigaura | 0.64 | 0.88 | 0.60 | 5.83 | 0.00 | 0.00 | 24.2 | 28.3 | 1.36 | 0.00 | 0.00 | 0.01 | 31.6 | 0.17 |
| Takatsuki   | 1.04 | 3.10 | 0.00 | 12.9 | 7.97 | 19.8 | 54.2 | 50.7 | 0.88 | 5.05 | 814 | 0.36 | 20.4 | 0.63 |

a World Reference Base for Soil Resources (FAO et al., 1998).

b Soil pH and electrical conductivity (EC) were determined in 1:5 w/v (soil:H2O) suspensions with a glass electrode.

c Total C and N contents were determined by the dry combustion method using an NC analyzer (Sumigraph NC–220F; Sumika Chemical Analysis Service).

d Soil texture was determined by the sieving method (sand content) and by the pipette method (silt and clay contents).

Water soluble form was analyzed in 1:5 w/v (soil:H2O) extractions for 1 h by inductively coupled plasma mass spectrometry (ICP–MS, Agilent 7700 Series; Agilent Technologies) to determine the concentrations of Na, Mg, Al, P, K, Ca, Mn, Fe, Cu, Zn, Rh, and Cs, and by a colorimetric method to determine the concentrations of NH4+ by the indophenol method (Keeney and Nelson, 1982) and NO3− by the method of Cataldo et al. (1975).
with tap water by an automatic irrigation system during the experiment with minimal drainage, and the excess drainage, if any, was captured with a bottom-closed container placed under the pot. During the flowering period, the blueberry bushes were cross-pollinated with another variety of rabbiteye blueberries ‘Tifblue’ with the aid of honey bees. During the experiment, the blueberry bushes were not pruned in order to avoid any loss of elements from the whole bushes.

Sampling and analysis of soil solution

The soil solution was collected by the suction method eight times as follows: in March (day 11), April (day 41), June (day 103 at flowering), and November (day 228 after harvest) in 2014, and in March (day 370), April (day 404), June (day 467 at flowering), and August (day 530 during harvest) in 2015 (Fig. 1, right).

To control the effect of soil moisture content on the concentration of the elements in the soil solution, we adjusted the soil water content to 60% of the maximum water-holding capacity (as determined separately prior to the experiment) by adding tap water the day before sampling. Within 24 h after watering, we collected less than 100 mL of the soil solution from each pot. The soil solution samples were kept frozen until analysis.

The soil solution was analyzed by inductively coupled plasma mass spectrometry (ICP–MS, Agilent 7700 Series; Agilent Technologies, Tokyo, Japan) to determine the concentrations of Na, Mg, Al, P, K, Ca, Mn, Fe, Cu, Zn, Rb, and Cs. The concentration of N was measured colorimetrically; NH₄⁺ by the indophenol method (Keeney and Nelson, 1982), and NO₃⁻ by the method of Cataldo et al. (1975). The pH of the soil solution was also measured with a glass electrode pH meter.

Sampling and analysis of fruit, leaves, branches plus stems, and roots

Matured fruit was sampled between 23 June and 30 October in 2014 and between 29 June and 17 September in 2015. The fruit was collected once a week. After counting the number and measuring the fresh weight, the samples were frozen at −30°C and then freeze-dried in a freeze dryer (FD-2MM, FDc-2BU; Nihon Techno Service, Ibaraki, Japan; FDU-2110; EYELA, Tokyo, Japan). The dried samples were weighed, ground into powder, and stored in desiccators until analysis.

After the final harvest of fruit on 17 September 2015, the bushes were divided into leaves, branches plus stems, and roots. The leaves were oven-dried at 70°C until constant weight. The branches plus stems were cut into 1-cm pieces and oven-dried. The roots were washed in tap water, cut into 1-cm pieces, and oven-dried. The oven-dried samples were weighed, ground into powder, and stored under ambient conditions until analysis.

The plant samples were subjected to wet digestion in concentrated HNO₃. The digested solutions were then analyzed by ICP–MS (Agilent 7700 Series; Agilent Technologies) to determine the concentrations of Na, Mg, Al, P, K, Ca, Mn, Fe, Cu, Zn, Rb, and Cs. The concentration of N was also measured by the dry combustion method using an NC analyzer (Sumigraph NC-220F; Sumika Chemical Analysis Service, Osaka, Japan). The content of the elements in each organ of the plants was calculated by multiplying the elemental concentration by the corresponding dry weight. The content in the whole bush was calculated by summing up the contents in all organs (fruit, leaves, branches plus stems, and roots).

Statistical analysis

Within each soil type, the results of the soil treatments with and without acidification and fertilization were compared with those of the control (no treatment) by one-way ANOVA. The effects of soil types and soil treatments on elemental concentrations in the soil solution and on elemental contents in the blueberry bushes were determined by two-way ANOVA. The effects of the soil solution pH on the concentration of the elements in the soil solution were also evaluated by correlation analysis. These statistical analyses were performed using Microsoft Excel 2010 software and Excel TOUKEI 2012 (Social Survey Research Information, Tokyo, Japan).

Fig. 1. The suction method for soil solution sampling. Two porous ceramic cups buried in the soil of each pot were connected with silicon tubes (left and middle) and vacuum syringes by which the soil solution was sucked and collected in the syringes (right).
Results and Discussion

Dynamics of pH and elemental concentrations in soil solution as influenced by soil type and soil treatment

Figure 2 shows the temporal changes in pH in soil solution during the experimental period. Under soil acidification alone, and in combination with fertilization, the soil solution pH decreased until 103 days, and then remained constant. This indicates that the applied sulfur was transformed into sulfates by microbial oxidation (Wainwright, 1984). Among the soil types with no treatment, Kasumigaura soil showed the lowest pH during the experimental period, which reflected the original soil pH (1:5 w/v) (Table 1). By acidification and its combination with fertilization, however, the pH of Takatsuki soil became the lowest. This is probably due to the smaller buffering capacity of Takatsuki soil than the other two soils.

Table 2 shows the average pH in soil solution during the whole experimental period. The combination of acidification plus fertilization resulted in a lower average soil solution pH than acidification alone in all soils. Fertilization also decreased the soil solution pH significantly compared with no treatment. This is probably due to the production of proton through nitrification of added ammonium (Barak et al., 1997).

The average elemental concentration in the root-zone soil solution showed that the dominant elements were Na, Mg, and Ca in all soils (Table 2). The concentrations of Na, P, Fe, and Cu in the soil solution were influenced by the soil type and were not changed by any soil treatments in any particular soil. Especially, P was 1/200–1/500 times lower in Tsukuba soil than in Takatsuki soil.

The concentration of elements in the soil solution was also influenced by the soil treatment (Table 2). Fertilization significantly increased NO$_3^-$ in all soils, but acidification alone and the combination with fertilization did not change in any soils, suggesting that the nitrification was limited under acidic conditions (Haynes and Swift, 1986). By fertilization, the addition of NH$_4^+$ and K to the soils significantly increased the average concentrations of not only NH$_4^+$ (in Tsukuba soil and Kasumigaura soil) and K (in all soils), but also NO$_3^-$ (in all soils), Ca (in all soils), Rb (in Tsukuba soil and Kasumigaura soil), and Cs (in Tsukuba soil and Kasumigaura soil) in the soil solution. Among these elements, the fertilization tended to increase the concentrations of the following elements: NH$_4^+$ (33–205 fold compared with no treatment), Cs (3.0–9.9 fold), NO$_3^-$ (2.1–8.4 fold), Rb (3.1–5.4 fold), K (3.4–4.7 fold), and Ca (2.9–4.5 fold). Comparing of the three soils, the NH$_4^+$, Rb, and Cs showed solubilization owing to fertilizer in Tsukuba soil and Kasumigaura soil, but Takatsuki soil did not. Our results agree in part with previous results showing that excess NH$_4^+$ and K in a soil caused the release of Mg, K, Ca, and Zn (Lorenz et al., 1994) and Cs (Chiang et al., 2008; Schulz et al., 1960) into the soil solution.

On the other hand, acidification caused a significant increase in the concentrations of Mg and Ca in all soil solutions (Table 2). The concentrations of Al (in Kasumigaura soil), P (in Kasumigaura soil and Takatsuki soil), Mn (in Tsukuba soil and Kasumigaura soil), Fe (in Tsukuba soil), Cu (in Kasumigaura soil), Zn (in Tsukuba soil and Kasumigaura soil), Rb (in Kasumigaura soil), and Cs (in Kasumigaura soil and Takatsuki soil) in the soil solution were also increased significantly by acidification. Among the elements, Al (77–1421 fold increase compared with no treatment) tended to be influenced more strongly by acidification than other elements; Zn (18–414 fold), Fe (1.2–204 fold), Mn (6.1–45 fold), Cu (2.4–16 fold), P (1.4–14 fold), Cs (1.9–8.1 fold), Mg (4.6–6.1 fold), Rb (2.2–5.6 fold), and Ca (2.3–4.9 fold). An increase in these elements following acidification was observed in

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**Fig. 2.** Temporal changes in pH in soil solution during the experimental period (548 days). Soil treatments: ◆ no treatment; ● acidification; ▲ fertilization; ■ acidification plus fertilization. The soil solution was sampled eight times: in March (day 11), April (day 41), June (day 103 at flowering), and November (day 228 after harvest) in 2014, and in March (day 370), April (day 404), June (day 467 at flowering), and August (day 530 during harvest) in 2015. Soil treatments (no treatment, acidification, fertilization, and acidification plus fertilization) were conducted at the beginning of the experiment. Fertilizer was applied a second time at the same application rate on day 377 of the experiment (30 March 2015). Error bars represent standard errors of the means (n = 3).
### Table 2. Average pH and elemental concentrations in soil solution during the experiments.

| Soil type       | Soil treatment          | pH     | NH₄⁺ (mg·L⁻¹) | NO₃⁻ (mg·L⁻¹) | Na (mg·L⁻¹) | Mg (mg·L⁻¹) | Al (mg·L⁻¹) | P (mg·L⁻¹) | K (mg·L⁻¹) | Ca (mg·L⁻¹) | Mn (mg·L⁻¹) | Fe (μg·L⁻¹) | Cu (μg·L⁻¹) | Zn (mg·L⁻¹) | Rb (μg·L⁻¹) | Cs (μg·L⁻¹) |
|-----------------|-------------------------|--------|---------------|---------------|-------------|-------------|-------------|------------|------------|-------------|-------------|--------------|--------------|-------------|-------------|-------------|
| Tsukuba         | No treatment            | 6.2 a  | 0.12 c        | 5.2 b         | 274 b       | 46 c        | 0.071 b     | 0.016 a    | 32 c       | 183 d       | 1.5 c       | 0.13 b       | 3.8 a        | 0.0080 c    | 12 c        | 0.094 c     |
|                 | Acidification           | 4.3 c  | 0.062 c       | 1.0 b         | 357 ab      | 213 ab      | 5.5 b       | 0.021 a    | 68 bc      | 427 c       | 30 b        | 22 a         | 8.9 a        | 0.14 b      | 27 bc       | 0.18 bc     |
|                 | Fertilization           | 5.8 b  | 4.1 b         | 43 a          | 389 ab      | 128 bc      | 0.047 b     | 0.0033 a   | 106 ab     | 541 b       | 0.87 c      | 0.039 b      | 6.2 a        | 0.012 c     | 37 b        | 0.28 b      |
|                 | Acidification plus Fertilization | 4.1 c | 8.3 a      | 13 b         | 468 a       | 294 a       | 14 a        | 0.015 a    | 149 a      | 666 a       | 68 a        | 14 a         | 9.1 a        | 0.26 a     | 60 a        | 0.54 a      |
| Kasumigaura     | No treatment            | 5.3 a  | 0.057 c       | 2.9 b         | 116 a       | 14 b        | 0.083 b     | 0.0041 b   | 10 c       | 38 b        | 8.2 b       | 11 a         | 4.9 b        | 0.011 b     | 7.3 d       | 0.058 d     |
|                 | Acidification           | 3.9 c  | 1.5 c         | 3.3 b         | 182 a       | 70 a        | 117 a       | 0.059 a    | 19 c       | 186 a       | 50 a        | 13 a         | 27 a         | 0.74 a      | 25 c        | 0.25 c      |
|                 | Fertilization           | 4.5 b  | 12 b          | 13 a          | 206 a       | 41 ab       | 0.51 b      | 0.0047 b   | 45 b       | 171 a       | 19 b        | 16 a         | 11 b         | 0.042 b     | 39 b        | 0.57 b      |
|                 | Acidification plus Fertilization | 3.7 c | 19 a      | 2.9 b       | 176 a       | 70 a        | 127 a       | 0.052 a    | 58 a       | 216 a       | 44 a        | 1.4 a        | 26 a         | 0.63 a     | 69 a        | 1.2 a       |
| Takatsuki       | No treatment            | 6.9 a  | 0.32 b        | 24 b          | 141 b       | 25 c        | 0.040 b     | 2.9 b      | 22 c       | 107 b       | 1.3 b       | 0.23 a       | 52 b         | 0.037 b     | 3.2 b       | 0.0073 c    |
|                 | Acidification           | 3.7 c  | 7.6 b         | 19 b          | 319 ab      | 152 ab      | 27 ab       | 6.7 a      | 48 bc      | 525 a       | 59 ab       | 46 a         | 845 ab       | 15 ab       | 18 b        | 0.059 b     |
|                 | Fertilization           | 6.1 b  | 12 b          | 50 a          | 230 ab      | 93 bc       | 0.054 b     | 3.0 b      | 84 ab      | 396 a       | 2.5 b       | 0.23 a       | 44 a         | 0.37 b     | 17 b        | 0.039 bc    |
|                 | Acidification plus Fertilization | 3.5 c | 39 a      | 16 b       | 345 a       | 243 a       | 53 a        | 7.2 a      | 128 a     | 584 a       | 115 a       | 46 a         | 1374 a       | 31 a       | 43 a        | 0.11 a      |

Means followed by the same letter within a column within a soil type are not significantly different at P<0.05 (Tukey's test; n=3).

*, ** Significantly different among soil types and soil treatments at * P<0.05 and ** P<0.01 (two-way ANOVA); ns, not significant.

### Table 3. Correlation coefficients between the average pH and elemental concentrations in soil solution during the experiments.

| Average concentration of elements in soil solution | NH₄⁺ | NO₃⁻ | Na | Mg | Al | P | K | Ca | Mn | Fe | Cu | Zn | Rb | Cs |
|--------------------------------------------------|------|------|----|----|----|---|---|----|----|----|----|----|----|----|
| Average soil solution pH                         | −0.51 ns | 0.49 ns | −0.29 ns | −0.53 ns | −0.62 * | −0.22 ns | −0.29 ns | −0.39 ns | −0.83 ** | −0.70 * | −0.48 ns | −0.50 ns | −0.63 * | −0.44 ns |

*, ** Significantly different among soil types and soil treatments at * P<0.05 and ** P<0.01, respectively; n=12.
Kasumigaura soil, but less so in the other soils. On the other hand, the concentration of Na and K in the soil solution did not change significantly by acidification in all soils.

The combination of acidification plus fertilization further increased the concentrations of the following elements in the soil solution compared with each treatment alone (Table 2); NH$_4^+$ (in all soils), Al (in Tsukuba soil), K (in Kasumigaura soil), Ca (in Tsukuba soil), Mn (in Tsukuba soil), Zn (in Tsukuba soil), Rb (in all soils), and Cs (in all soils).

Table 3 shows correlation coefficients between the average pH and elemental concentrations in the soil solution. The analysis was performed across all soil types and treatments. The effects of the soil solution pH on the elemental concentration in the soil solution showed significant negative correlations between soil solution pH and Al ($P<0.05$), Mn ($P<0.01$), Fe ($P<0.05$), and Rb ($P<0.05$). Among the 13 elements examined, the soil solution acidification had a stronger influence on the release of Al, Mn, Fe, and Rb from the soil solid phase to the solution phase.

**Fruit yield, fresh fruit weight, and dry weight of blueberry bushes as influenced by soil type and soil treatment**

Table 4 shows the fruit yield, fresh fruit weight, and dry weight of the organs of the blueberry bushes. The fruit yield did not differ significantly by soil type or treatment in either year. In both years, the fresh fruit weight was influenced by soil type and by soil treatment, but the interaction was not significant across all soil types and treatments. In the first year, the fresh fruit weight in each treatment alone significantly decreased in Takatsuki soil. In the second year, acidification plus fertilization significantly decreased fresh fruit weight in Kasumigaura and Takatsuki soils, although in Tsukuba soil, fresh fruit weight did not differ significantly by treatment in either year. In Kasumigaura soil, the decrease in fresh fruit weight resulted from the increase in the number of fruits that exceeded the increase in fruit yield. In Takatsuki soil, the decrease in fresh fruit weight accounted for a decrease in the fruit yield, but no changes in the fruit number by acidification plus fertilization. The fruit dry weight did not differ significantly by soil type or by treatment, but was significantly influenced by the interaction across all soil types and treatments. The dry weights of leaves and of branches plus stems differed significantly by soil treatment, but did not differ significantly by soil type. The dry weight of roots did not differ significantly by treatment within each soil type, but was overall significantly influenced by soil type. The total bush weight was significantly increased by fertilization, and acidification plus fertilization in Tsukuba and Kasumigaura soils, but was not significantly increased in Takatsuki soil, indicating that the bushes in Takatsuki soil did not grow well in fertilized conditions. The total bush weight was significantly

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**Table 4.** The fruit yield, fresh fruit weight, and dry weight of the organs of the blueberry bushes.

| Soil type   | Soil treatment               | Fruit yield* (g FW/bush) | Fresh fruit weight* (g FW/fruit) | Dry weight* (g/bush) |
|-------------|------------------------------|--------------------------|----------------------------------|----------------------|
|             | 1st year        | 2nd year        | 1st year        | 2nd year        | Fruit | Leaves | Branches plus stems | Roots | Total |
| Tsukuba     | No treatment    | 325 a           | 628 a           | 1.2 a           | 1.3 a | 92 a | 25 b       | 150 ab | 104 a | 371 c |
|             | Acidification   | 282 a           | 721 a           | 1.1 a           | 1.2 a | 109 a | 17 b       | 132 b  | 115 a | 373 bc|
|             | Fertilization   | 327 a           | 743 a           | 1.0 a           | 0.98 a| 104 a | 39 ab      | 217 a  | 123 a | 483 ab|
|             | Acidification plus Fertilization | 227 a | 759 a | 1.1 a | 1.0 a | 129 a | 51 a | 185 ab | 136 a | 501 a |
| Kasumigaura | No treatment    | 381 a           | 504 a           | 1.3 a           | 1.5 a | 77 a  | 15 b       | 130 b  | 135 a | 357 c |
|             | Acidification   | 371 a           | 745 a           | 1.2 a           | 1.2 ab| 120 a | 21 b       | 155 ab | 126 a | 422 bc|
|             | Fertilization   | 335 a           | 813 a           | 1.2 a           | 1.2 ab| 139 a | 58 a       | 176 a  | 145 a | 518 a |
|             | Acidification plus Fertilization | 356 a | 887 a | 1.1 a | 1.0 b | 137 a | 44 a | 175 a  | 138 a | 495 ab|
| Takatsuki   | No treatment    | 461 a           | 935 a           | 1.2 a           | 1.1 a | 143 a | 29 b       | 156 b  | 91 a  | 420 b |
|             | Acidification   | 300 a           | 645 a           | 0.83 b          | 1.0 ab| 100 a | 24 b       | 149 b  | 116 a | 388 b |
|             | Fertilization   | 387 a           | 589 a           | 0.89 b          | 0.95 ab| 84 a  | 55 a       | 217 a  | 104 a | 460 a |
|             | Acidification plus Fertilization | 316 a | 721 a | 0.97 ab | 0.84 b | 104 a | 35 ab | 171 b  | 125 a | 435 ab|

* Fruit yield and fresh fruit weight were measured in both years.

* Dry weight was measured only in the second year.

* FW: fresh weight.

Means followed by the same letter within a column within a soil type are not significantly different at $P<0.05$ (Tukey’s test; n = 3).

*, ** Significantly different among soil types and soil treatments at * $P<0.05$ and ** $P<0.01$ (two-way ANOVA); ns, not significant.
affected by soil treatment and the interaction across all soil types and treatments, but not by soil type.

**Elemental absorption by blueberry bushes and the distribution of elements in blueberry organs**

Table 5 shows the elemental contents in whole blueberry bushes as influenced by soil type and soil treatment. The content of Al in the blueberry bushes was high enough to be comparable to the contents of N, Na, K, and Ca among the elements examined in this study. Although the concentration of Al, Fe, and Cs in the soil solution was significantly affected by soil type, soil treatment, or the interaction (Table 2), the contents of these elements in the blueberry bushes were not significantly changed by any soil treatment in each soil type. The effects of soil treatment on the content of elements in blueberry bushes are summarized as follows: (1) fertilization, and the combination of acidification plus fertilization, resulted in significant increases in N (in all soils) and K (in all soils), and in Mg (in Tsukuba soil and Takatsuki soil) and P (in Takatsuki soil); (2) acidification enhanced the significant increases in Mn (in all soils), and in K (in Kasumigaura soil and Takatsuki soil), Cu (in Takatsuki soil), Zn (in Takatsuki soil), and Rb (in Kasumigaura soil and Takatsuki soil); and (3) fertilization, but not acidification, resulted in significant increases in N (in all soils), and in Na (in Tsukuba soil and Takatsuki soil), Mg (in Tsukuba soil and Takatsuki soil), P (in Takatsuki soil), K (in Tsukuba soil), and Ca (in Tsukuba soil and Kasumigaura soil).

Beside the soil treatment, the soil type also influenced the contents in the blueberry bushes (Table 5). The contents of N, Na, Mg, Al, P, Mn, Fe, Cu, Zn, Rb, and Cs in the bushes were significantly affected by soil type ($P < 0.05$). Of these 11 elements, the contents of Al, Fe, Rb, and Cs were highest in bushes grown in Kasumigaura soil, while the contents of the seven other elements (N, Na, Mg, P, Mn, Cu, and Zn) were higher in bushes grown in Takatsuki soil. The bushes in Kasumigaura soil contained the highest Al concentration in the roots (approximately 10488 mg·kg$^{-1}$), but the dry weight of the roots was not significantly reduced (Table 4). The Al concentration levels in the roots of blueberry bushes as influenced by soil type and soil treatment, or the interaction across all organs. More than 40% of Al, Fe, Cu, and Cs were located in the roots. In particular, more than 70% of Al and Fe were located in the roots, and the percentages were extremely low in the other organs (fruit, leaves, and branches plus stems) (<6% for Al and <15% for Fe).

Iron and Cu have been recognized to accumulate in blueberry roots, and are extremely high in acidic soil conditions (Bagatto and Shorthouse, 1991; Katakura and Hirota, 2003). In addition, there are previous findings for Al accumulation in blueberries grown in acidic soils (e.g., Katakura and Hirota, 2003). Accumulation of Al in the roots may be partly due to the contamination of soil particles in the root samples because Al is the third most abundant element following O and Si in agricultural soils in Japan (Yanai et al., 2012) and because washing in tap water could not completely separate the roots from soil particles. Despite the possible artifacts, the concentration of Al in blueberry roots (2745–10488 mg·kg$^{-1}$) in our study was comparable to or higher than the concentration in roots of blueberry bushes grown in other soils (approximately 4000–8000 mg Al·kg$^{-1}$; Katakura and Hirota, 2003) or in hydroponic solutions containing different Al concentrations (approximately 200–3500 mg·kg$^{-1}$; Reyes-Díaz et al., 2010). The Al content in roots increased in parallel with the Al concentration in hydroponic solutions up to 100 μmol·L$^{-1}$ in ‘Legacy’, but at concentrations above 100 μmol·L$^{-1}$, the Al content in the roots of ‘Bluegold’ remained constant (Reyes-Díaz et al., 2010). Moreover, higher soil acidity did not increase the translocation of Al and Fe from the roots to aboveground parts (Katakura and Hirota, 2003). These findings indicate that blueberry roots have mechanisms to retard the absorption of Al and also the translocation to the aboveground parts.

**Relationship between solubilization of elements in root zone soil and their elemental absorption by blueberry bushes**

In Figure 3, the content of each element in the blueberry bushes was compared with its average concentration in the soil solution. The contents of Na, Mg, Al, K, Ca, Mn, Fe, Zn, and Rb in the bushes were all greater than the initial contents, indicating active absorption of these elements during the experiment. Across all soil types and treatments, the contents of N, P, K, Mn, Cu, and Zn in the blueberry bushes showed significant positive correlations ($P < 0.01$) with the average concentration of the corresponding element in the soil solution, whereas seven other elements (Na, Mg, Al, Ca, Fe, Rb, and Cs) did not, partly due to the lower requirement than their supply from the soil. For P, Cu, and Zn showing significant correlations, the high values of Takatsuki soil plotted in Figure 3 made the correlation coefficients higher. This suggested that the soil type had a stronger influence on the P, Cu, and Zn availability for the blueberry bushes.
### Table 5. Elemental contents in blueberry bushes as influenced by soil type and soil treatment.

| Soil type          | Soil treatment          | N  (mg/bush) | Na  (mg/bush) | Mg  (mg/bush) | Al  (mg/bush) | P  (mg/bush) | K  (mg/bush) | Ca  (mg/bush) | Mn  (mg/bush) | Fe  (mg/bush) | Cu  (mg/bush) | Zn  (mg/bush) | Rb  (mg/bush) | Cs  (mg/bush) |
|--------------------|-------------------------|--------------|---------------|---------------|---------------|--------------|--------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| **Tsukuba**        | No treatment            | 1163 b       | 341 b         | 241 c         | 461 a         | 265 a        | 1134 b       | 888 b         | 42 c          | 127 a         | 0.76 b        | 7.5 a         | 0.65 b        | 0.020 a       |
|                    | Acidification           | 1082 b       | 215 b         | 249 bc        | 468 a         | 257 a        | 1287 b       | 783 b         | 149 b         | 191 a         | 1.2 ab        | 7.7 a         | 0.84 ab       | 0.015 a       |
|                    | Fertilization           | 2081 a       | 690 a         | 342 ab        | 1121 a        | 361 a        | 1625 a       | 1439 a        | 43 c          | 269 a         | 1.1 ab        | 9.5 a         | 0.73 ab       | 0.018 a       |
|                    | Acidification plus Fertilization | 2032 a      | 480 ab        | 375 a         | 1291 a        | 330 a        | 1774 a       | 1196 ab       | 224 a         | 513 a         | 2.0 a         | 11 a          | 1.0 a         | 0.035 a       |
| **Kasumigaura**    | No treatment            | 947 b        | 342 ab        | 270 b         | 1441 a        | 244 a        | 1038 c       | 919 b         | 85 c          | 580 a         | 1.6 a         | 7.2 b         | 1.1 c         | 0.044 a       |
|                    | Acidification           | 1203 b       | 238 b         | 298 ab        | 1379 a        | 286 a        | 1322 b       | 868 b         | 144 ab        | 627 a         | 1.9 a         | 9.0 ab        | 2.2 a         | 0.058 a       |
|                    | Fertilization           | 1957 a       | 410 ab        | 330 ab        | 1491 a        | 301 a        | 1710 a       | 130 bc        | 491 a         | 1.8 a         | 10 ab         | 1.5 bc        | 0.038 a       |
|                    | Acidification plus Fertilization | 2198 a      | 585 a         | 370 a         | 1128 a        | 345 a        | 1779 a       | 1068 ab       | 478 a         | 2.2 a         | 11 a          | 1.9 ab        | 0.046 a       |
| **Takatsuki**      | No treatment            | 1534 b       | 643 b         | 269 c         | 334 a         | 356 b        | 1189 c       | 1029 ab       | 42 c          | 110 a         | 1.4 b         | 9.1 c         | 0.48 b        | 0.017 a       |
|                    | Acidification           | 1777 b       | 488 b         | 327 bc        | 717 a         | 454 ab       | 1391 b       | 903 b         | 169 b         | 513 a         | 7.8 a         | 29 b          | 1.2 a         | 0.035 a       |
|                    | Fertilization           | 2578 a       | 1104 a        | 362 ab        | 313 a         | 522 a        | 1672 a       | 1371 a        | 45 c          | 106 a         | 1.9 b         | 12 c          | 0.47 b        | 0.010 a       |
|                    | Acidification plus Fertilization | 2553 a      | 795 ab        | 424 a         | 895 a         | 517 a        | 1705 a       | 1157 ab       | 232 a         | 456 a         | 8.5 a         | 55 a          | 1.0 a         | 0.034 a       |

Means followed by the same letter within a column within a soil type are not significantly different at $P<0.05$ (Tukey’s test; $n=3$).

*, ** Significantly different among the soil types and the soil treatments at * $P<0.05$ and ** $P<0.01$ (two-way ANOVA); ns, not significant.

### Table 6. Elemental contents in fruit, leaves, branches plus stems, and roots as a percentage of the total in blueberry bushes.

| Soil type | Soil treatment | N (%) | Na (%) | Mg (%) | Al (%) |
|-----------|----------------|-------|--------|--------|--------|
| **Tsukuba** | No treatment | 20    | 16     | 36     | 28     |
|           | Acidification | 23    | 14     | 31     | 32     |
|           | Fertilization | 19    | 19     | 36     | 26     |
|           | Acidification plus Fertilization | 21    | 22     | 28     | 29     |
| **Kasumigaura** | No treatment | 15    | 11     | 31     | 43     |
|           | Acidification | 22    | 15     | 30     | 33     |
|           | Fertilization | 22    | 23     | 25     | 29     |
|           | Acidification plus Fertilization | 26    | 20     | 25     | 28     |
| **Takatsuki** | No treatment | 25    | 18     | 29     | 28     |
|           | Acidification | 19    | 16     | 25     | 40     |
|           | Fertilization | 14    | 25     | 34     | 28     |
|           | Acidification plus Fertilization | 18    | 18     | 28     | 36     |
| Soil type   | Soil treatment       | P (%) | K (%) | Ca (%) | Mn (%) |
|------------|----------------------|-------|-------|--------|--------|
| Tsukuba    | No treatment         | 17    | 4.7   | 43     | 36     |
|            | Acidification        | 21    | 3.6   | 34     | 42     |
|            | Fertilization        | 18    | 5.9   | 41     | 35     |
|            | Acidification plus Fertilization | 20 | 8.6 | 34 | 37 |
| Kasumigaura | No treatment       | 15    | 3.0   | 32     | 50     |
|            | Acidification        | 20    | 3.9   | 33     | 43     |
|            | Fertilization        | 20    | 9.4   | 28     | 43     |
|            | Acidification plus Fertilization | 23 | 6.7  | 29  | 42 |
| Takatsuki  | No treatment         | 23    | 4.9   | 41     | 32     |
|            | Acidification        | 19    | 3.6   | 33     | 44     |
|            | Fertilization        | 15    | 7.3   | 45     | 33     |
|            | Acidification plus Fertilization | 20 | 5.0  | 33  | 42 |

| Soil type   | Soil treatment       | Fe (%) | Cu (%) | Zn (%) | Rb (%) | Cs (%) |
|------------|----------------------|--------|--------|--------|--------|--------|
| Tsukuba    | No treatment         | 1.3    | 5.3    | 8.0    | 85     | 16     |
|            | Acidification        | 1.0    | 2.8    | 3.8    | 92     | 15     |
|            | Fertilization        | 0.90   | 6.3    | 5.5    | 87     | 15     |
|            | Acidification plus Fertilization | 0.74 | 4.5  | 2.9  | 92 |
| Kasumigaura | No treatment       | 0.26   | 0.9    | 1.3    | 98     | 7.9    |
|            | Acidification        | 0.59   | 2.1    | 2.2    | 95     | 10     |
|            | Fertilization        | 0.63   | 4.0    | 2.3    | 93     | 9.6    |
|            | Acidification plus Fertilization | 0.79 | 2.9  | 2.6  | 94 |
| Takatsuki  | No treatment         | 2.3    | 9.8    | 9.3    | 79     | 9.5    |
|            | Acidification        | 0.21   | 2.5    | 2.1    | 95     | 3.1    |
|            | Fertilization        | 0.84   | 15     | 11     | 73     | 6.7    |
|            | Acidification plus Fertilization | 0.32 | 2.7  | 2.7  | 94 |

n = 3
Fig. 3. Relationships between the content of each element in blueberry bushes and the average concentration in the soil solution (n = 12). Soil treatments: ◤ no treatment; ● acidification; ▲ fertilization; ■ acidification plus fertilization. Soils: black, Tsukuba; blue, Kasumigaura; red, Takatsuki. The concentration of N in the soil solution was calculated by summing the concentrations of NH$_4^+$ and NO$_3^−$. The dashed line indicates the content in the blueberry bushes at the beginning of the experiment (24 March 2014, n = 2).
Comparing among the elements showing significant correlations, the slope of the regression equation decreased in the following order; P (29) > N (25) > Cu (5.7) > K (4.5) > Mn (1.9) > Zn (1.4) (Fig. 3). The steep slopes of the regression equation of P and N suggested that intensive replenishment from the soil solid phase had occurred to meet the requirements of the blueberry bushes.

In conclusion, soil acidification and/or fertilization, together with the original soil type, significantly influenced the concentration of many elements in the soil solution. Across all soil types and treatments, the average concentrations of N, P, K, Mn, Cu, and Zn in the soil solution showed significant positive correlations with the content of the corresponding element in blueberry bushes, which suggests the importance of soil solution analyses as a real-time soil test for better soil management.

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