Boron isotopes reveal differences in boron uptake in tomato plants from different sources

Troy Rasbury, Katie Wooton, Brooke Peritore, Carrie Wright, Melissa Passik, Rebekah Wooton, Shannon Letscher, Corey Wong, Aidan Clarke, Michael Doall

With changing climate and sea levels, growing populations, increasing pollution and other environmental perturbations, humans need to adapt and find sustainable alternatives to current modes of living. While agriculture is critical for food security, some practices are nutritionally inefficient and/or introduce an unsustainable burden on the environment. One of the elements needed for plant growth and satisfactory agricultural yields is boron. While boron is an essential element for plants, it is toxic if concentrations are too high. Deficiency is equally problematic. Between deficiency and toxicity, there is only a narrow window (Brdar-Jokanović, 2020). Few studies have focused on boron isotopes to understand how plants uptake boron.

We wanted to explore boron isotope inclusion in terrestrial agricultural plants, to determine details of uptake, through a controlled growth experiment that involved supplying plants with different sources and concentrations of boron. Building on our experiments from summer 2020 (Peritore et al., 2021), we grew 6 tomato plants in pots from July 7, 2021 to October 20, 2021 (105 days). These plants were cared for and documented by high school students Corey Wong (Mt Sinai HS) and Aidan Clarke (Ward Melville HS). These researchers keep a record of the watering history, took daily photographs of the plants, and weekly took samples of leaves, as well as flowers and fruit when available. A control plant had only potting soil. Two plants were fertilized with un-composted seaweed, but the seaweed was layered in the pots in different ways. One plant had a liquid boron supplement. One plant had a boron supplement in the form of pellets. One plant was fertilized by manure. The plants were purchased at the same time and from the same flat, so they started out with identical controls.

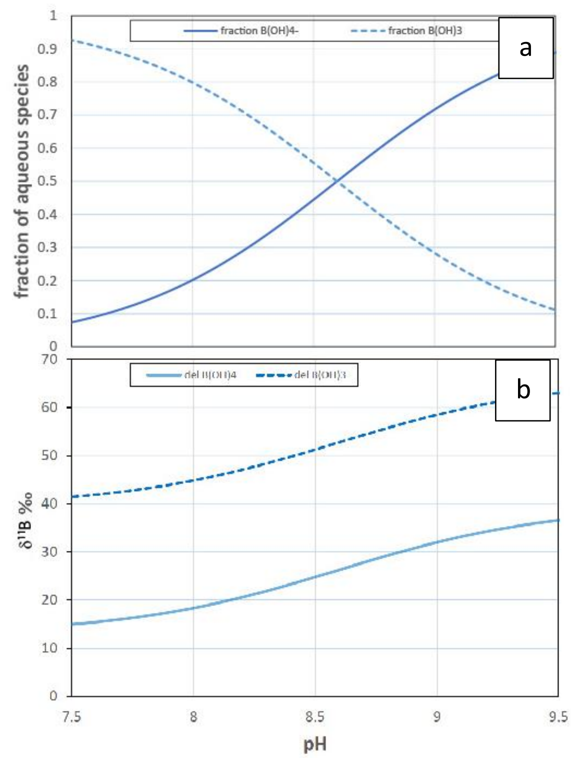

Figure 1a: Boron speciation with pH. Boric acid (dashed curve) dominates at low pH, while borate (solid blue curve) dominates at elevated pH. **1b:** Because there is an isotope fractionation between species, the proportion of the species controls the isotope compositions (modified from Hemming and Hanson, 1992).

Boron isotopes and systematics

Boron has two isotopes, ^{11}B and ^{10}B. In solution, boron has two primary species, $B(OH)_3$ (boric acid) and $B(OH_4)^-$ (borate). There is a pH control on the speciation, and the species have different affinities for the boron isotopes such that there is a fractionation between them (Hemming and Hanson, 1992). The fractionation factor in seawater has been determined experimentally to be 26.2‰ (Klochko et al., 2006). At low pH boric acid is dominant, while at high pH borate is dominant (Fig. 1). The fractionation between species and the proportion of species in solution, results in differences in the isotope composition of the species that is controlled by pH (Fig. 1). In most soils (pH ~5-8), boric acid dominates and contains ≥80% of the boron (Fig. 1). The offset in borate $\delta^{11}B$ from the reservoir is most extreme in the pH range of most soils, making the $\delta^{11}B$ of the resulting plant a proxy for which species is being taken into the plant. If the process were indiscriminate, it is anticipated that the $\delta^{11}B$ would be that of the soil/fertilizer, and because boric acid is such a large fraction of the total boron, it would have near identical values to the soil fertilizer. In earlier research, we have found isotopically light boron in seaweed with an average of 17‰ (Wright et al., 2021), while the seawater value is 39.6‰ (Foster et al., 2010). Others have shown extremely light boron in diatoms (Armbrecht et al., 2018; Donald et al., 2020). Combined, these results suggest that primitive plants and phytoplankton select for borate. Tomatoes, peppers, and basil grown with seaweed as a fertilizer showed a trend in boron isotopes towards the $\delta^{11}B$ of seaweed (Peritore et al., 2021). The experiments presented here build on that observation.

Methods

A flat of tomato plants was purchased from a local nursery. Roots, soil, and leaves were sampled for boron analyses from the seedlings. Using the same potting soil (Vigoro All Purpose) we planted with a range of amendments to the soil. The amendments used were liquid boron (Agricultural Boron), boron pellets (Cameron pellets), seaweed (*Saccharina latissimi*, or sugar kelp, which was cultivated along longlines in Moriches Bay, NY. The kelp was harvested in May and dried in a greenhouse, and stored until use in this experiment), and manure (Bovung from Home Depot). The plants were grown outside in pots and were watered as needed but also received rainwater. Tap water and rainwater have low boron concentrations (Peritore et al., 2020) and are not expected to influence the experiment. Photos were taken of the plants daily, and leaf, flower and fruit samples were taken weekly.

After collection, the samples were dried and then weighed for analyses. The samples were leached with 2% nitric acid. 1 ml of nitric for flowers, 2mL of nitric for leaves and 3mL of nitric for tomatoes was added to 15 ml centrifuge tubes and were leached over days to weeks. Aliquots of 0.5-2.5 mls were taken from the leachates and ammonia was used to adjust solutions to pH ~9. The pH adjusted samples were put through Amberlite boron specific resin to concentrate boron and remove all other ions from the samples. The boron was eluted in 2% nitric acid and then diluted for signal matching to 50 ppb NBS 951 (boric acid standard). The samples were run by Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC ICPMS). Standards and samples are bracketed by nitric blanks and the average boron signal from the bracketing nitric blanks is subtracted from the standards and samples. The $\delta^{11}B$ of the samples is calculated by reference to the NBS 951 boric acid standard which is taken as 0‰.

TABLE 1: Fertilizers and Initial composition

Sample	$\delta^{11}B$	2SD
Dead Tomato Leaf	-14.78	0.02
Tomato Roots	-1.16	0.94
Original leaf	-13.50	1.21
Saccharina latissimi	17.00	2
Bovung Manure	19.70	0.76
Bonnie Plants Soil	12.26	1.00
Vigoro All Purpose	11.32	1.00
Agricultural Boron	-13.4	0.8
Cameron pellets	-8.9	0.6

Results

The potting soil that the plants came in, has a similar $\delta^{11}B$ to that purchased for the project (Vigoro All Purpose; hereafter called Vigoro) at about 11‰ (Table 1). This contrasts with the roots and initial leaf analyses which gave $\delta^{11}B$ values of -1.16 and -13.5 (Table 1). The manure and seaweed are isotopically heavier than the potting soil, but distinct from each other with manure being the isotopically heaviest material analyzed (Table 1). The boron supplements, both of which are from inorganic borate minerals have isotopically light boron (Table 1).

Control Tomato Plant

The control plant was planted in Vigoro potting soil without other amendments. The plant started turning yellow about a week earlier than the plants that were fertilized with seaweed. Within the first week, the $\delta^{11}B$ of the leaves jumped to 20‰, and the leaves remained above 20‰ for 75 days (Fig. 2). The final three samples declined toward the potting soil value as concentrations also declined (Fig. 2). The flowers and tomatoes show a similar trend (Fig. 3). There are significant offsets between the leaves and tomatoes, that appear to switch when the trends change. Minimum boron concentrations in the leaves correspond to the light $\delta^{11}B$ values (Fig. 4). The leaves have lower boron than the flowers and the fruit has very low concentrations.

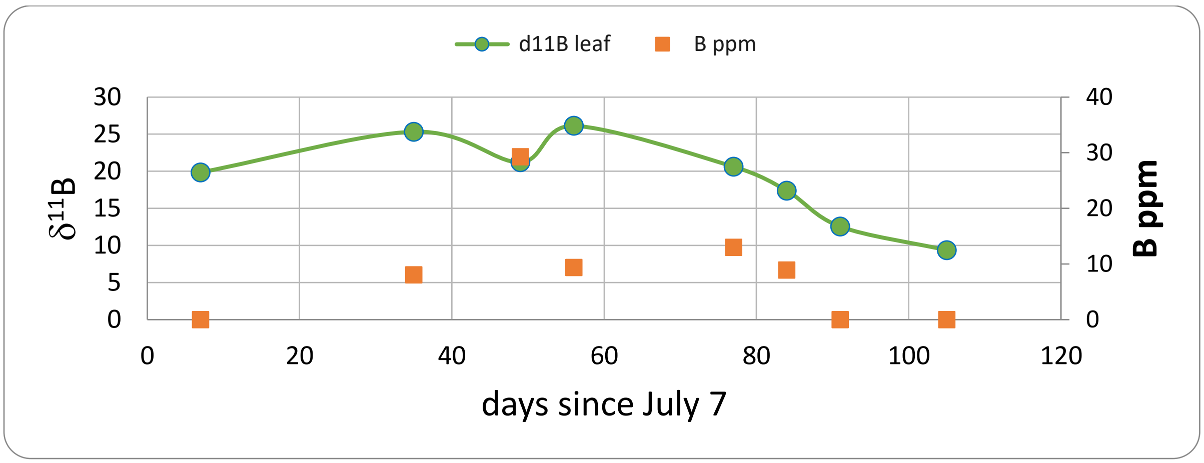

Figure 2: Control plant leaf boron isotope composition and concentrations.

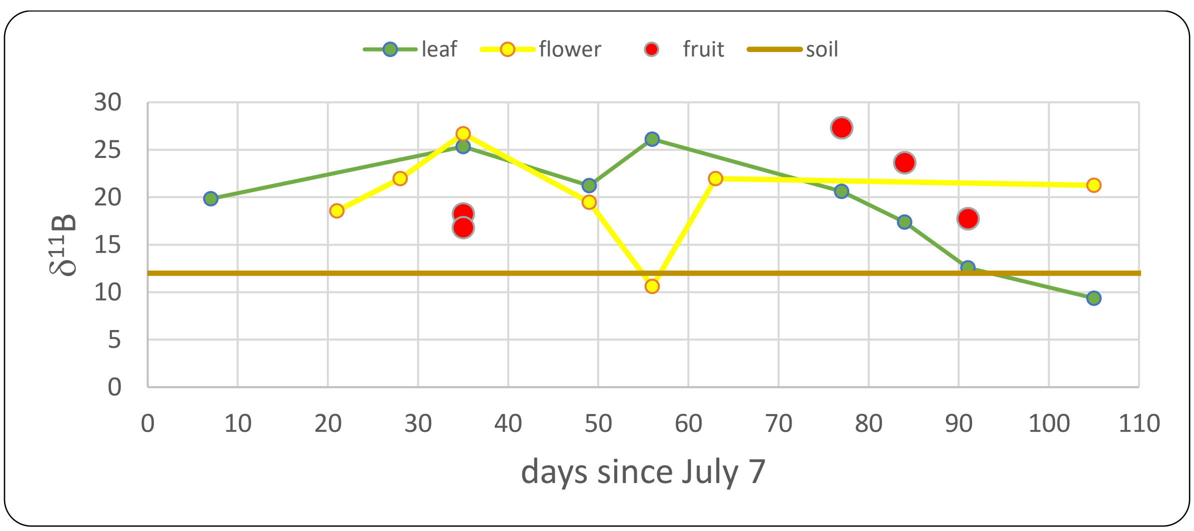

Figure 3: Boron isotope results for the leaves, flowers and fruit of the control tomato plant.

Seaweed Fertilized Plants

In one of the seaweed pots layered from bottom to top (bottom-1.5in seaweed, 2in soil, 1.5in seaweed, 3in soil). Another plant was layered from bottom to top (2in soil, 1in seaweed, soil (2 in), seaweed (1 in), soil (3 in). Both plants grew similarly and had about the same amount of fruit. Both plants showed a marked leap to heavier $\delta^{11}B$ in the first week, followed by a steady decline to the seaweed value by 80 days, where the $\delta^{11}B$ crosses to lower than seaweed values (Fig. 4, Fig. 5).

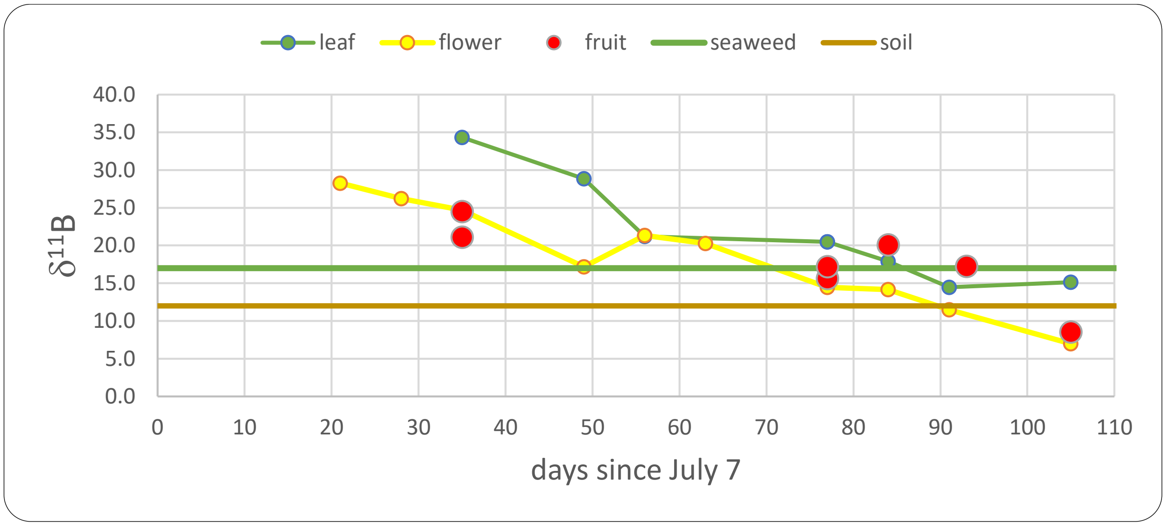

Figure 4: Tomato plant planted in potting soil with seaweed added as a fertilizer on the bottom and interlayered throughout the planting pot.

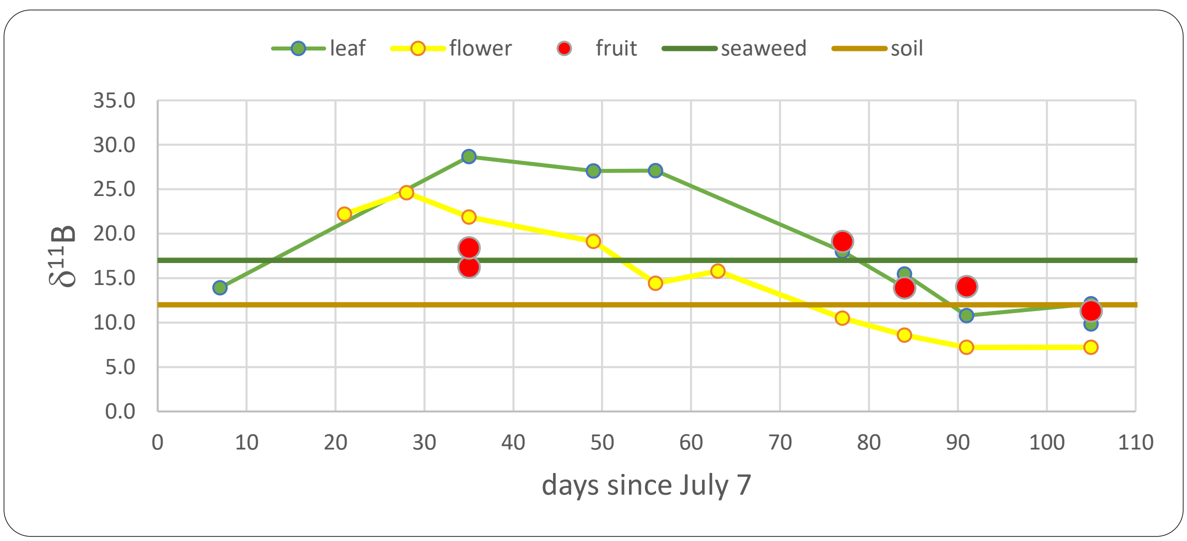

Figure 5: Tomato plant planted in potting soil with soil on the bottom and seaweed layered throughout the planting pot.

Boron Supplement Plants

As with the control and seaweed fertilizer plants, the first leaves collected from the tomato plants with inorganic boron supplements, showed an initial marked increase in $\delta^{11}B$ from the value of the initial leaves (Fig. 6, Fig. 7). However, neither of these tomato plants came close to the potting soil composition. The initial plant in the liquid boron supplement died quickly, due to toxicity, within hours with a $\delta^{11}B$ of

-14.78‰ (Table 1). To compensate for this, we took a small part of the liquid boron infused potting soil from the dead plant's pot and mixed it with fresh potting soil so that the boron was diluted by a factor of ten or so. After an initial rise, the δ^{11}B declined to ≤-40‰ after the first month and stayed around -40‰ for the remainder of the summer (Fig. 6). This plant was less than a foot tall and did not produce flowers (or fruit). The leaves from the early stages of growth have far more boron than the isotopically light boron through most of the summer.

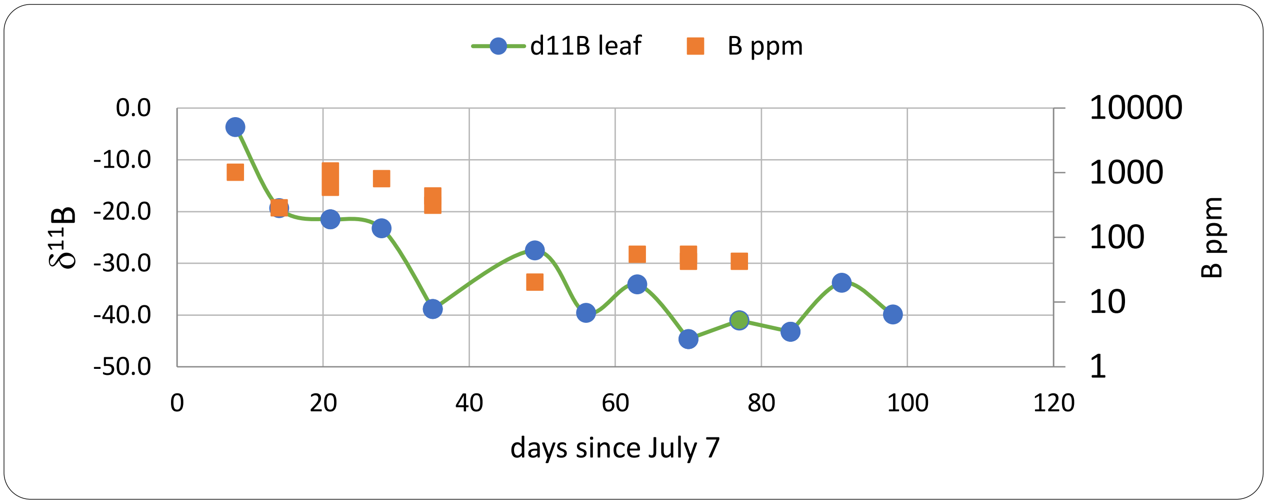

Figure 6: Boron in tomato with liquid boron supplement. This plant did not produce flowers or fruit.

The tomato plant with the boron pellet supplement had δ^{11}B values mostly between those of the soil and the supplement (Fig. 7). This plant produced flowers, but little fruit. The flowers show a significant difference from the δ^{11}B of the leaves after 60 days (Fig. 7).

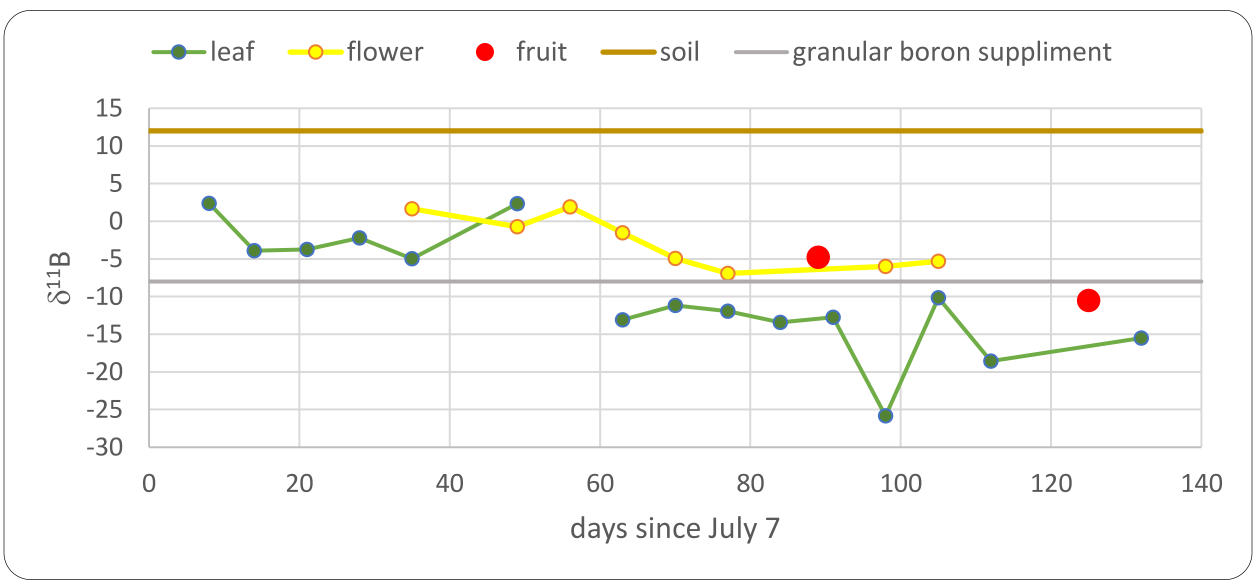

Figure 7: Boron isotopes for the tomato plant with borate granules added as supplement.

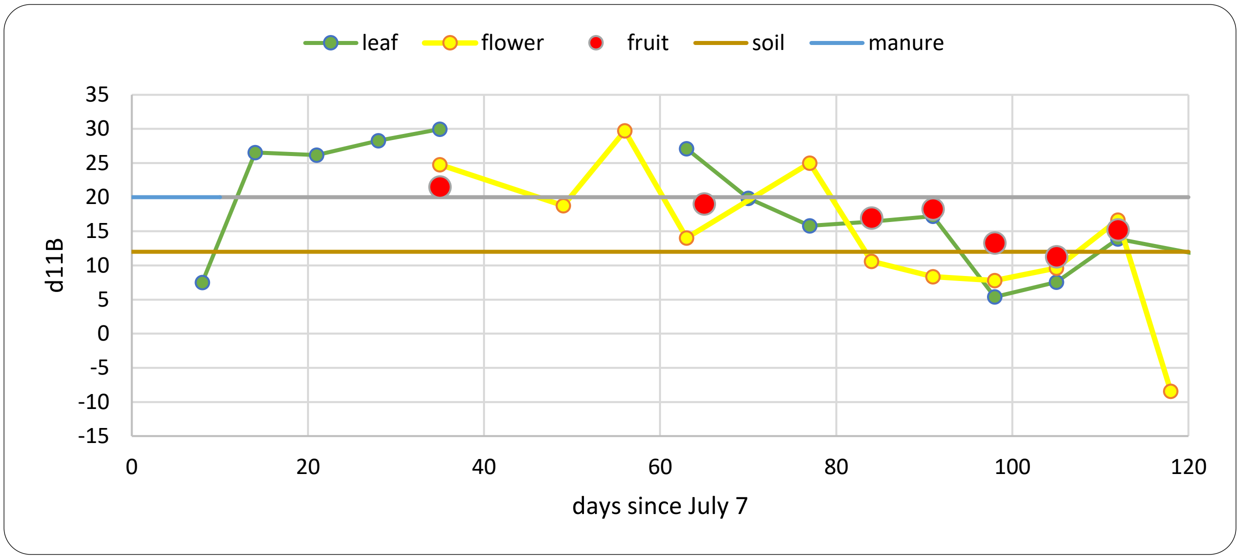

Figure 8: Boron isotopes in a tomato planted with manure.

Discussion

Our survey of six tomato plants with a variety of fertilizers show substantial differences in the uptake of boron based on concentration and isotope ratio values. Since the plants and soils have the same starting conditions and are treated the same way, the differences must be in the behavior of the supplements, perhaps reflecting how boron becomes available to the plant. A root sample, which was taken when we transplanted the tomatoes had a $\delta^{11}B$ of -1.16‰, while the initial leaf had a $\delta^{11}B$ of -13.5‰ (Table 1). Because the -13.5‰ value is anomalous with respect to the roots and to the original soil values, we suspect if may have been contaminated by the very high concentration liquid boron supplement with that isotope signature, but the root values demonstrate that the initial boron was isotopically light. The control plant which was grown with only potting soil stands out in that the $\delta^{11}B$ of all but the very last leaf collected is elevated over the value of the potting soil it grew in (Fig. 2). The plant that was fertilized with manure shows a similar pattern, though after two months the $\delta^{11}B$ declines to below manure values and after three months declines below soil values (Fig. 8). The two plants with seaweed as a fertilizer jump up initially to more than 20‰ but then decline through the rest of the summer to values that, by day 80, are lower than seaweed (Fig. 4; Fig. 5).

In general, the control and plants with natural supplements (manure and seaweed) show similarities in that they initially jump to higher values and then decline towards the reservoir value with time. These trends could suggest that the plant is preferentially taking up boric acid such that the remaining reservoir becomes isotopically lighter. The flowers in these plants are mostly isotopically lighter than the leaves at the same stage. The tomato fruit has isotope ratios that are like that of the flowers. In terms of concentrations there is a general trend of higher concentrations in the leaves than the flowers, and in the flowers than the fruit (as shown for the control plant in Fig. 3). The trend of decreasing $\delta^{11}B$ from the leaves to the flowers suggests a fractionation within the plant. However, the direction of this change is opposite to that seen in a study of boron in bell pepper plants (Geilert et al., 2019, 2015). Since our plants were in a small pot and nothing was added after the initial fertilization, it is possible the changes we see in $\delta^{11}B$ through time reflect depletion of boron in the reservoir.

Similar to the control and naturally fertilized plants, the tomatoes with inorganic boron supplement addition showed a sharp increase followed by a decline in the $\delta^{11}B$. The plant with granular boron, which dissolves slowly had $\delta^{11}B$ values mostly greater than the boron supplement. In fact, these values were fairly stable and could be explained as a mix between the soil and the boron pellets (Fig. 7). The last two leaf analyses did dip to values lower than the supplement. In contrast, the plant with liquid fertilizer declined dramatically to values of ≤-40‰ within a few weeks. These values are consistent with borate derived from a solution with an initial composition of the supplement (-13.4‰) at a pH of 6 (Fig. 9), which is the pH we measured for the potting soil with the supplement added. In this plant it is quite clear that the plant is selecting for borate.

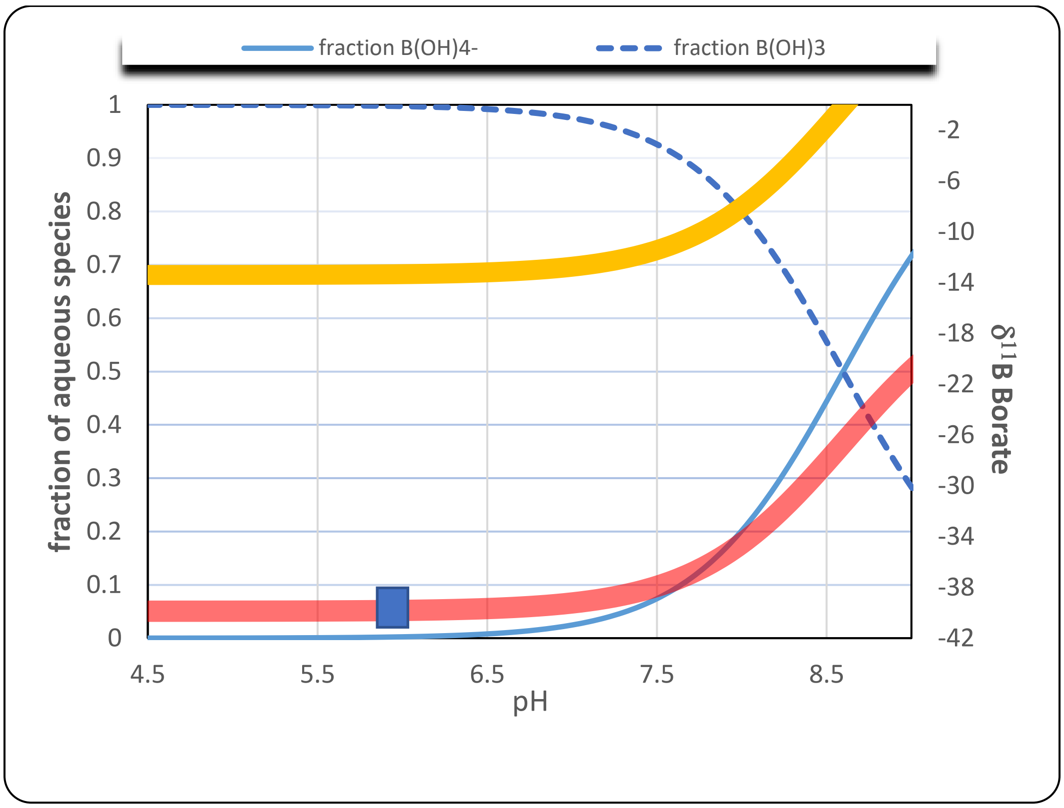

Figure 9: Boron speciation as shown in Fig. 1 but modified for the soil system we are examining. The $\delta^{11}B$ of the supplement is -13.4. The blue box represents the pH of the soil and the isotope composition of the plant leaves.

Taken together, the declining values of all the plants through the experiment may result from a preferential uptake of borate into the plant. For all the plants, the fact that the values jump up suggests that the earliest boron released and available to the plants is isotopically heavy. We do not know what that is, but we suspect it is in the soil itself. In terms of what species the plant is taking up, the results from the plant with liquid boron supplement are clearcut, while the other plants show differences which we suggest shows that the boron supplements are behaving differently. That is, the boron that is available to go into the plant with the liquid supplement is available right away and it is just a question of adding water and allowing the boron species to form and fractionate boron isotopes (Fig. 9). In the case of the boron pellets, we would suggest that there is a slow release (these pellets do not dissolve quickly in the lab) and that the reservoir of boron that the plant taps into may have a composition between the soil and the supplement. The control, seaweed, and manure show similar patterns that suggest that lighter boron is becoming available through the breakdown of the supplements. Future experiments will start with seeds so we can better understand the initial conditions and why we see the big jump at the beginning of this experiment.

Conclusions

In terms of the health and productivity of the tomato plants, the plant with seaweed and manure produced the greatest number of tomatoes (best agricultural yield), and the plants with the inorganic boron supplement produced little and no fruit. Current studies by the Gobler Lab in SoMAS are evaluating how seaweed cultivation can help improve coastal water quality by extracting excess nitrogen and other nutrients from the water, which can lead to eutrophication, harmful algae blooms, and low oxygen. . Once the kelp is harvested, a major initiative is to determine ways to use it that supports sustainability. Seaweed has been used as a fertilizer for millennia, and the results from our tomatoes suggest that it can be used without composting. Thus, we suggest that experiments that build on using seaweed as a natural fertilizer could have the dual impact of nutrient bioextraction in coastal waters while enriching soils with a fertilizer that is not subject to excessive leaching into the environment. Future work will focus on using soils other than potting soils which have added supplements to have a better understanding of how the supplement we add is incorporated into the plant.

Bibliography

Armbrecht, L.H., Lowe, V., Escutia, C., Iwai, M., McKay, R., Armand, L.K., 2018. Variability in diatom and silicoflagellate assemblages during mid-Pliocene glacial-interglacial cycles determined in Hole U1361A of IODP Expedition 318, Antarctic Wilkes Land Margin. Mar. Micropaleontol. 139, 28–41. https://doi.org/10.1016/j.marmicro.2017.10.008

Brdar-Jokanović, M., 2020. Boron Toxicity and Deficiency in Agricultural Plants. Int. J. Mol. Sci. 21, 1424. https://doi.org/10.3390/ijms21041424

Donald, H.K., Foster, G.L., Fröhberg, N., Swann, G.E.A., Poulton, A.J., Moore, C.M., Humphreys, M.P., 2020. The pH dependency of the boron isotopic composition of diatom opal (<i>Thalassiosira weissflogii</i>). Biogeosciences 17, 2825–2837. https://doi.org/10.5194/bg-17-2825-2020

Foster, G.L., Pogge von Strandmann, P.A.E., Rae, J.W.B., 2010. Boron and magnesium isotopic composition of seawater. Geochem. Geophys. GEOSYSTEMS 11. https://doi.org/10.1029/2010GC003201

Geilert, S., Vogl, J., Rosner, M., Eichert, T., 2019. Boron isotope variability related to boron speciation (change during uptake and transport) in bell pepper plants and SI traceable $n(^{11}B)/n(^{10}B)$ ratios for plant reference materials. Rapid Commun. Mass Spectrom. 33, 1137–1147. https://doi.org/10.1002/rcm.8455

Geilert, S., Vogl, J., Rosner, M., Voerkelius, S., Eichert, T., 2015. Boron isotope fractionation in bell pepper. Mass Spectrom. Purif. Tech. 1, 101.

Hemming, N.G., Hanson, G.N., 1992. Boron isotopic composition and concentration in modern marine carbonates. Geochim. Cosmochim. Acta 56, 537–543. https://doi.org/10.1016/0016-7037(92)90151-8

Klochko, K., Kaufman, A.J., Yao, W., Byrne, R.H., Tossell, J.A., 2006. Experimental measurement of boron isotope fractionation in seawater. Earth Planet. Sci. Lett. 248, 276–285. https://doi.org/10.1016/j.epsl.2006.05.034

Peritore, B., Downs, D., Wooton, K., Rasbury, T., 2020. Isotopic Evidence in Rainwater for Boron Contribution in Long Island Groundwaters, in: Geology of Long Island and Metropolitan New York. Presented at the 27th Conference, Stony Brook.

Peritore, B., Downs, D., Wright, C., Wooton, K., Doall, M., Rasbury, T., 2021. Boron in Soils, Fertilizers and Plants in a Pilot Garden Study, in: 28th Conference on the Geology of Long Island and Metropolitan New York. Presented at the Long Island Geologists, Stony Brook University.

Wright, C.C., Wooton, K.M., Twiss, K.C., Newman, E.T., Rasbury, E.T., 2021. Boron Isotope Analysis Reveals Borate Selectivity in Seaweeds. Environ. Sci. Technol. 55, 12724–12730. https://doi.org/10.1021/acs.est.1c02860