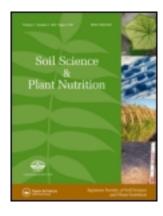
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Potential for Using Plant Xylem Sap to Evaluate Inorganic Nutrient Availability in Soil

II. Comparison between the Xylem Sap Method and the Extraction Method

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The "extraction method" that is commonly used to evaluate the availability of nutrients in soil often involves complex, problematic and simultaneous assays of multiple components and comparisons of analytical values. In a previous paper using pot experiments of plants, we confirmed the possibility of using nutrient concentrations in the xylem sap of Luffa cylindrica Roem. seedlings as an indicator of nutrient availability in soil and suggested that the "xylem sap method," might represent a solution of the above-mentioned problems and inaccuracies characteristic of chemical extraction. To determine whether a method enables to evaluate available nutrient contents in soil, the criterion should probably be that the relationship between the values obtained by that method and the amounts of nutrients absorbed by plants does not vary depending on the types of soil. In this regard, we compared the conventional extraction method with our proposed xylem sap method. In the present paper, we attempted to change the cation exchange capacity (CEC) in soil by the addition of zeolite and/or humic acid to soil to change the nutrient availability without changing the total quantity of nutrients in soil in the pots. Our results showed that the amounts of nutrients absorbed by plants changed under these treatments. However, in some cases, changes in the amounts of phosphorus, calcium, magnesium, and zinc obtained via different extractants did not correspond to the changes in the amounts of nutrients absorbed by the plants. Thus, the amounts of nutrients absorbed by the plants were compared with nutrient concentrations in the xylem sap and the amounts of nutrients inferred by each extractant to examine their relationship. A highly positive correlation was consistently found between the amounts of nutrients absorbed by the plants and their concentrations in the xylem sap for every nutrient. By contrast, there was a lower correlation for the extraction method with some extractants. For phosphorus in particular, no close correlation was obtained between the amounts of nutrients absorbed by the plants and the amounts extracted, regardless of the extractants. In the present study, because we attempted to change the nutrient availability by artificially controlling the soil CEC value, without changing the total amount of nutrients present in the culture medium, the changes in nutrient availability were not conspicuous. Thus, it is possible that the extraction method itself generates large errors for such small changes. Therefore, we concluded that the xylem sap method is superior to the extraction method, as an indicator of nutrient availability in soil, at least under the conditions of the present experiment.

Key Words: availability, CEC, inorganic nutrients, Luffa cylindrica, xylem sap method.

To prevent waste of fertilizer resources and reduce environmental pollution due to excessive fertilization, fertilization must be kept to the minimum required to replace specific missing nutrients in arable soil and to satisfy the nutrient requirements of crops. For this reason, the available soil nutrients should be precisely determined. Numerous studies on the identification of element availability in soil have been conducted recently on a certain kind of fertilizer component (Cox et al. 1999; Magdoff et al. 1999), on a certain kind of plant (Köhler et al. 2001; Zhang and George 2002; Guillard and Dest 2003), and on heavy metal availability for the evaluation of toxicity in soils contaminated with heavy metals (Rooney et al. 1999; Conder et al. 2001; Ayoub et al. 2003). However, to manage the productivity of arable soil appropriately, it is necessary to obtain comprehensive information about the "multiple components" that plants require.

Currently, the "extraction method" is commonly used for evaluating the contents of available nutrients in soil. In this method, soil is shaken with an appropriate solution and the amounts of nutrients extracted are used as an indicator (Marschner 1995). Although this method has several advantages, including convenience of use, it is also associated with several shortcomings that should be overcome, including the difficulty in simultaneous evaluation of multiple components (Noguchi et al. 2005). To address these problems, we proposed the "xylem sap method" in a previous paper (Noguchi et al. 2005), which the nutrient concentrations in the xylem sap are used to evaluate the contents of available nutrients in soil. It was eventually concluded that the nutrient concentrations in the xylem sap could be used as an indicator to evaluate the contents of available nutrients in soil, based on the following three findings obtained from experiments in which Luffa cylindrica Roem. seedlings were grown in test soil: 1) changes in the nutrient concentrations in the xylem sap accurately reflected the changes in the available nutrient concentrations in the rhizosphere solution; 2) the nutrient contents in the xylem sap also reflected the nutritional status of the plant; and 3) changes in the nutrient concentrations in the xylem sap were found to correspond to the availability of nutrients in soil and to changes in their availability.

To determine whether a method is effective in evaluating the contents of available nutrient in soil, it is important that the relationship between the amounts of nutrients measured using the method and the quantity of nutrients absorbed by the plant, the growth and yield of the plant, or deficiency/excess of nutrients, remains constant, regardless of the type of soil. However, the growth and yield of a plant are not determined only by the rate of intake of one kind of nutrient. Also, deficiency or excess does not always occur. Therefore, it is reasonable to simplify our criterion, such that, regardless of the type of soil, the relationship between the amounts of nutrients measured in soil using the method and the amounts of nutrients absorbed by the plant does not change.

Based on our experience, the extraction method frequently generates a non-proportional relationship between the values obtained and the amounts of nutrients absorbed by the plant. For example, although no increase/decrease is observed in the values obtained by extraction method, the amounts of nutrients the absorbed by the plant may have increased/decreased. We have also observed the reverse. Because the assumed extraction conditions are affected by the physical and chemical properties of the test soil, it is likely that they cannot be standardized. For example, when shaking a soil while adding to it an extractant solution at a given pH, the pH of the soil itself can cause the pH of the extractant to deviate from the optimal range during the extraction. It is, therefore, desirable to develop an evaluation method in which the relationship with the amounts of nutrients absorbed by a plant can be applied to different soil types.

Therefore, we compared the xylem sap method with the extraction method in term of the evaluation of the nutrients available in soil, assuming the existence of a linear relationship between the latter and their increased or decreased absorption by the plant.

Nutrient availability in soil is influenced by the physical and chemical properties of the soil, such as pH, cation exchange capacity (CEC), concentrations of coexisting substances, in addition to the abundance of nutrients. In particular, differences in the CEC value of a soil may change the cation concentrations in the soil solution, exerting a direct influence on the availability of nutrients present as cations. The CEC value of soil can be altered through human intervention. For example, application of organic matter to fields to enhance fertilizer retentivity to allow timed absorption by plants is recommended. In recent years, studies have been under taken to examine the application of zeolite as a heavy metal adsorbent in soils contaminated with heavy metals as well as an inhibitor of heavy metal absorption by plants (Singh et al. 2000; Oste et al. 2002). In the present study, the CEC value of the test soil was artificially varied using humus and clay, and L. cylindrica seedlings were grown in this CEC-controlled soil. The amounts of nutrients absorbed by the plant were then compared with the amounts of nutrients extracted from soil with the extractants and their concentrations in the xylem sap to examine their relationship. Based on our results, we then determined whether the xylem sap method is a suitable method for evaluating the nutrient availability in soil.

MATERIALS AND METHODS

Controlling the soil CEC. Two kinds of materi-

Table 1. Addition of zeolite and humic acid to 200 g of Gray Lowland soil.

Treatment	Zeolite	Humic acid	CEC of soil made up	
	g (200	$(\text{cmol}_{c} \text{ kg}^{-1})$		
Α	0.0	2.0	20	
В	0.4	1.0	20	
С	0.8	0.0	20	
D	0.0	20.0	45	
Е	4.0	10.0	45	
F	8.0	0.0	45	

als were used to change the CEC value of soil: the humic acid reagent (Code No.; 082-04625, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and synthetic zeolite (Code No.; 263-00575, "Zeolite, Synthetic, A-3, Beads", Wako Pure Chemical Industries, Ltd., Osaka, Japan). Both zeolite and humic acid were sieved through a 0.15 mm screen, with the zeolite being crushed beforehand. Humic acid and/or zeolite were added to 200 g of Gray Lowland soil with a CEC value of 17.5 cmol_{c} kg⁻¹ which was used in our previous experiments (Noguchi et al. 2005), as shown in Table 1, to prepare six kinds of test soils: CEC values of the soil prepared were 20 cmol, kg^{-1} for A, B and C and 40 cmol kg^{-1} for D, E and F (CEC values of sieved humic acid and zeolite were $270-320 \text{ cmol}_{c} \text{ kg}^{-1}$ and $645-730 \text{ cmol}_{c} \text{ kg}^{-1}$, respectively).

Cultivation. A CEC-controlled soil sample (200 g of Gray Lowland soil and humic acid and/or zeolite) was blended with 200 g of quartz sand (grain size: 0.5-1 mm), and placed in a 500 ml plastic pot. Six cotyledonous *Luffa cylindrica* Roem. cv. Futo-Hechima seedlings per pot grown in nursery beds filled with nonfertilized vermiculite were transplanted to the pots. After transplantation, another 70 g of aliquot quartz sand was added on top of the soil. The seedlings were grown in a greenhouse under natural light and regularly watered with deionized water. Pot weight was monitored to maintain 60% of the maximum water-holding capacity of the soil once a day at least. The soil was not fertilized. The seedlings were grown in five replicates.

Collection of xylem sap. The collection protocol followed that described in our previous paper (Noguchi et al. 2005). After the test plants had been cultivated to the four-leaf stage (or for 20 d), the stem was cut at the level of the hypocotyl below the cotyledons (approximately 1 cm above the ground), and the part above the cut was removed. One end of a soft plastic tube was fitted over the end of the cut hypocotyl, with the other end leading to a test tube. Xylem sap was collected continuously for 24 h, starting in the morning.

Extraction of nutrients from soil. Following the collection of the xylem sap, the soil in which plants

had been grown was air-dried and sieved through a 0.5 mm mesh screen to remove quartz sand and roots. From this test soil, with a uniform grain size, elements were extracted according to the following procedure, and analytical samples were prepared.

Water extraction: Fifty milliliters of distilled water was added to 5 g of dried test soil. The suspension was shaken for 2 h and filtered (Kuboi et al. 1989). The filtrate was used as an analytical sample.

Sulfuric acid extraction: Four hundred milliliters of a 0.001 M sulfuric acid solution (pH 3) was added to 2 g of dried test soil. The suspension was shaken for 30 min and filtered (Nanzyo 1986). The filtrate was used as an analytical sample. This method was used for the extraction of phosphorus (Truog method).

Ammonium acetate extraction: Fifty milliliters of a 1 M ammonium acetate solution (pH 7) was added to 5 g of dried test soil. The suspension was shaken for 1 h and filtered (Hyuga 1981; partially modified). The filtrate was used as an analytical sample. This method was used for the extraction of potassium, calcium, magnesium, manganese, zinc, and copper.

Ammonium nitrate extraction: Fifty milliliters of a 1 M ammonium nitrate solution was added to 5 g of dried test soil (Symonides and McRae 1977; Kuboi et al. 1989). The suspension was shaken for 1 h and filtered. The filtrate was used as an analytical sample.

Hydrochloric acid extraction: Fifty milliliters of a 0.1 M hydrochloric acid solution was added to 10 g of dried test soil (Shibuya et al. 1978). The suspension was shaken for 1 h and filtered. The filtrate was used as an analytical sample.

EDTA extraction: Twenty milliliters of a 0.05 M EDTA (ethylenediaminetetraacetic acid) solution at pH 7 was added to 5 g of dried test soil. The suspension was shaken for 2 h. The supernatant was obtained after centrifugation at 2,000 rpm for 15 min. The decanted supernatant was evaporated to dryness and thermally decomposed by the addition of 1 ml of sulfuric acid, 5 ml of nitric acid, and 20 ml of perchloric acid. After decomposition was completed, the acids were evaporated almost to dryness. The total volume was made up to 50 ml with 0.1 M hydrochloric acid and the resultant mixture was used for analytical samples (Nagai and Yamanouchi 1975; Symonides and McRae 1977).

Analysis. The phosphorus concentration in the extraction samples of nutrients from the xylem sap and soil was measured by the molybdenum blue colorimetric method (micro-flow spectrophotometer UV-730; Shimadzu Corporation, Kyoto, Japan), and the potassium, calcium, magnesium, manganese, zinc, and copper concentrations were measured in the same samples by atomic absorption spectrometry (atomic absorption spectrophotometer A-2000; Hitachi, Ltd., Tokyo, Japan). The removed and dried shoots of the plants were subjected to wet decomposition using nitric acid and hydrogen peroxide, and their nutrient contents were measured using the above-described colorimetric and atomic absorption spectrometric methods.

RESULTS AND DISCUSSION

Changes in the plant growth and volume of the xylem sap exuded are shown in Figs. 1 and 2, respectively. Differences in the soil CEC values and blending ratios between humic acid and zeolite did not affect appreciably the shoot weight, root weight or the amount of xylem sap exuded by of the plants. No changes were observed between the treatments in term of leaf color or other external aspects of the plants. We therefore concluded that there were no changes in the nutrient availability in soil that would be sufficiently significant to influence the growth or the physiological status of the plants.

Table 2 shows the nutrient contents in the shoots. Because there were no differences in growth among the treatments, differences in the nutrient contents could be considered to be proportional to their level of absorption. The calcium, magnesium, manganese, zinc, and copper contents were lower in soils with a higher CEC value (ABC>DEF), presumably because, the quantity

of cations adsorbed onto soil increased with the increase of the CEC value in soil. Consequently, under the experimental conditions where no cations were supplied externally, their abundance in available form decreased, such that it is likely that the amount of absorption also decreased. Of these elements, the manganese and zinc contents in the shoots were lower in soils with a higher zeolite proportion (A > B > C, D > E > F), probably because, for the same CEC value, these elements were more easily adsorbed onto zeolite than onto humic acid. Although a similar tendency was observed for calcium and magnesium, it was not as prominent as with manganese and zinc. Conversely, the copper content was lower in soils with a higher humic acid proportion (A < B < C, $D \le E \le F$). This was ascribed to the fact that copper is able to form chelate compounds with humus, so that its availability would decrease with an increase in the blending proportion of humic acid. Although the phosphorus content was higher in soils with a higher CEC value, the difference was not conspicuous. In addition, no obvious influence of the blending ratio of humic acid and zeolite on the phosphorus content was observed. This is probably because the availability of phosphorus, which occurs as an anion in soil, is not related to the changes in the cation adsorption capacity of the soil. A large amount of potassium was absorbed from a higher proportion of zeolite, or at a higher CEC value of soil. It is likely that the synthetic zeolite used in the present

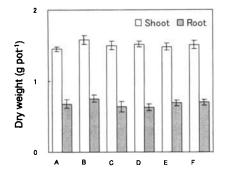


Fig. 1. Relationship between soil CEC value and plant growth. For symbols A–F, see Table 1. Bars indicate standard deviation.

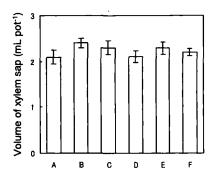


Fig. 2. Relationship between soil CEC value and xylem sap exudation. For symbols A–F, see Table 1. Bars indicate standard deviation.

	Table	2.	Nutrient	contents	in	plant	shoots.
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Treatment -	Р	К	Ca	Mg	Mn	Zn	Cu
	(mg kg ⁻¹)						
Α	1,330	22,000	10,800	4,580	1,230	304	22.0
В	1,270	23,800	11,100	4,640	779	273	26.8
С	1,250	25,400	9,000	4,070	435	210	26.2
D	1,330	23,700	5,950	3,370	893	185	13.6
E	1,480	28,100	6,040	3,450	269	124	13.1
F	1,490	30,600	5,370	3,350	211	72	17.4

Nutrient	Extractant	Treatment					
		Α	В	С	D	Е	F
Р	Water	12.4	12.3	12.2	10.3	11.1	10.3
	Sulfuric acid	237	269	259	218	199	86
	Hydrochloric acid	996	1,020	989	913	742	686
К	Water	316	354	384	330	391	422
	Ammonium acetate	376	451	513	416	945	1,610
	Hydrochloric acid	1,110	1,460	1,640	1,280	3,200	4,080
Ca	Water	93.0	74.0	38.7	74.5	23.1	10.3
	Ammonium acetate	1,150	1,220	1,220	1,220	1,060	970
	Hydrochloric acid	2,760	2,350	2,180	2,830	2,070	657
Mg	Water	51.3	45.0	38.7	54.3	23.4	15.6
	Ammonium acetate	346	346	344	372	279	207
	Hydrochloric acid	560	539	408	607	433	195
Mn	Water	30.2	21.5	14.3	21.3	9.9	8.3
	Ammonium acetate	132	120	103	101	66	34
	Hydrochloric acid	420	377	265	363	312	104
Zn	Water	4.23	2.44	1.10	2.64	2.06	1.05
	Ammonium acetate	17.0	18.2	13.8	10.8	6.6	3.6
	Hydrochloric acid	105	96	90	110	93	58
Cu	Water	0.06	0.13	0.18	0.03	0.04	0.28
	Ammonium acetate	2.49	2.51	2.76	1.05	0.98	2.26
	Hydrochloric acid	30.3	31.4	29.6	12.2	12.0	13.4

Table 3. Amounts $(mg kg^{-1})$ of nutrients extracted from soil with various extractants.

experiment contained potassium, so that the addition of zeolite to soil led to increased potassium levels. It is, therefore, possible that the changes in potassium availability might depend on the total amount of zeolite in soil, rather than on the soil CEC.

The amounts of nutrients extracted from soil were, in increasing order of the amounts extracted: water<ammonium acetate, ammonium nitrate<EDTA and hydrochloric acid. Table 3 shows the amounts of nutrients extracted from soil using water, which extracted the smallest amount, ammonium acetate, medium amount, and hydrochloric acid, the largest amount. The values of phosphorus extracted represent those when sulfuric acid was used (Truog method).

The amounts of calcium, magnesium, manganese, zinc, and copper extracted were lower in soil with a higher CEC value (ABC>DEF). However, the soil CEC value did not exert a remarkable effect on the amount of zinc extracted by hydrochloric acid. The amounts of calcium, magnesium, manganese, and zinc extracted were low when the blending ratio of zeolite increased (A>B>C, D>E>F). However, the amounts of calcium and magnesium extracted with ammonium acetate were hardly influenced by the blend ratio of humic acid and zeolite when the soil CEC value was 20 cmol_c kg⁻¹. Although ammonium acetate is generally used as an extractant to indicate the content of an exchangeable form, i.e. the indicator of effectiveness of these elements, in the present study, it did not necessarily give results corresponding to those of calcium and magnesium absorption by the plants. The influence of the blending ratio of humic acid and zeolite on the amount of copper extracted was not clear. The amount of phosphorus extracted was relatively, but not absolutely small when a soil with a high CEC was used. This is the opposite of the results for the phosphorus content in the plants. The amount of potassium extracted was larger when a soil with a higher CEC value and a higher zeolite proportion was used, presumably due to the additional potassium present in zeolite.

Provided that the values obtained by the extraction method accurately reflect the nutrient availability in soil (and not simply the nutrient content), the amounts of nutrients extracted should display changes similar to those in the nutrient contents in the plants associated with differences in either the soil CEC value or humic acid/zeolite blending ratio, as described above. The relation between the changes in the amounts of nutrients extracted and the changes in the nutrient contents in the plants described above was not consistent, in some cases.

Thus, we examined which of the two methods was more appropriate as an indicator of nutrient availability in soil by comparing the relationship of the nutrient con-

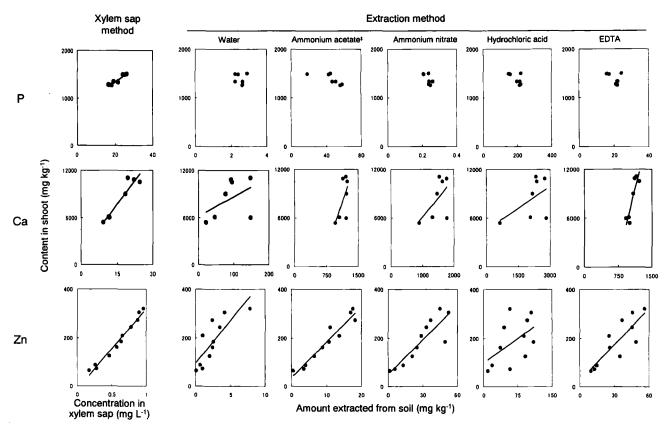


Fig. 3. Relationship of P, Ca, and Zn concentrations in xylem sap and amounts of elements extracted using each extractant with their contents in the plants, respectively. Top row: P, middle row: Ca, and bottom row: Zn. [‡]P was extracted with sulfuric acid instead of ammonium acetate.

tent in the plants with the concentration of each of the nutrients in the xylem sap and the values obtained by each extraction method. The results for phosphorus (an anion), calcium (a macronutrient), and zinc (a micronutrient) are shown in Fig. 3.

No significant correlation was found between the amount of phosphorus extracted with each extractant, including sulfuric acid (Truog method), and the measurement of the content in the plants. Phosphorus, occurring in soil as an anion, should show small changes in availability based on changes in the soil CEC values. For such small changes, the error range of the extracted amount should be large, suggesting that there was a limit to the range of evaluation by the extraction method. The phosphorus concentration in the xylem sap and the phosphorus content in the plants were, however, directly proportional even for the small changes in contents observed, indicating that minimal changes in soil can be identified using the phosphorus concentration in xylem sap as an indicator. The calcium and zinc contents of the plants were directly proportional to both the extracted amounts with all the extractants and the concentrations of these elements in the xylem sap. In particular, the relationship with their concentrations in the xylem sap was most closely proportional, as indicated by the fact

that the results from all the plots converged to an almost straight line. The same results were obtained with potassium, magnesium, manganese, and copper (data not shown). Therefore, based on the observed correspondence with the increasing or decreasing relationship in the amounts of nutrients absorbed by plants, it appears that the concentrations in the xylem sap are a very good indicator of any extracted amount.

To further clarify this assumption, correlation coefficients were calculated between the content of each nutrient in the plants and the corresponding concentration in the xylem sap, and the amount extracted. The results are shown in Fig. 4. A consistently high correlation (with a coefficient of nearly 1) was found between the content in the plants and the concentration in the xylem sap for every nutrient. By contrast, for many elements, such a consistently high correlation was not observed between the extracted amounts with all the extractants and contents in the plants. Phosphorus showed no particular correlation with the content in the plants for any of the extractants. However, for all the extractants, potassium showed a relatively high correlation with the content of the plants for both the concentration in the xylem sap and the extracted amount.

In the present study, the total amount of nutrients in

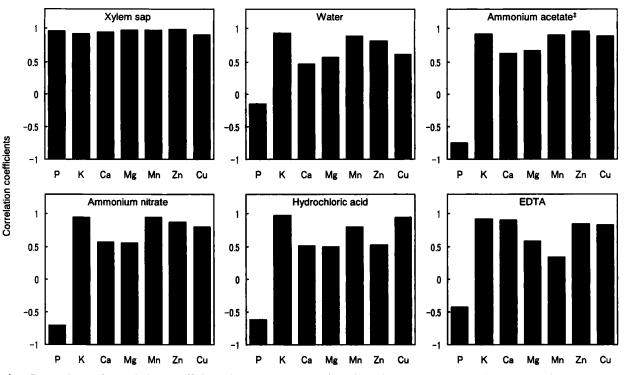


Fig. 4. Comparison of correlation coefficients between concentrations in xylem sap or extracted amounts with extractants and nutrient contents in the plants. [‡]P was extracted with sulfuric acid instead of ammonium acetate.

soil did not change. For changes in nutrient availability that are not very conspicuous, the nutrient concentrations in the xylem sap clearly changed correspondingly, but the extraction method could generate unrelated values in some cases.

An advantage of the xylem sap method is its ability to extract multiple nutrients simultaneously using a single method. Because the method does not require different extraction procedures depending on the type of soil or element to be analyzed, it not only facilitates comparisons among the analytical values, but also increases efficiency. Furthermore, because the soil does not need to be air-dried before testing it, the method can be applied for the evaluation of the availability of nutrients whose form changed owing to soil oxidation.

Our method ensured that a sufficient amount of xylem sap could be collected using L. cylindrica seedlings that had been grown for approximately 20 d after transplantation. With conventional extraction methods, several days are required for air-drying and sieving soil samples and several more for extracting nutrients. Thus, more than 2 weeks might be required until the extraction is completed when a large number of soil samples and nutrients are to be analyzed with this method. Therefore, we consider that the 20-d requirement of the xylem sap method for the growth period may not be unduly long. If the number of elements to be analyzed is small, the amount of xylem sap needed will be small, allowing an even shorter growth period. Furthermore, the use of graphite furnace atomic absorption spectrometry and ICP may enable to use diluted xylem sap for the analysis. In such a case, a smaller amount of xylem sap will also be sufficient.

In the present method, we used L. cylindrica as an extraction tool instead of conventional extractants to extract available nutrients in soil: the nutrients in soil were absorbed by L. cylindrica to generate the nutrient concentrations in the xylem sap, which were used to determine the available contents of nutrients. We will not deal with the nutrient absorption properties of L. cylindrica as a species because the L. cylindrica plant was only used as a tool in the present study. The advantage of the xylem sap method lies in its ability to extract several nutrients simultaneously from soil without the need for air-drying of soil. However, further verification might be necessary by accumulating more data on the relationship between the concentrations of available nutrients in the rhizosphere and their concentrations in the xylem sap. Such verification would include the growth of plants in various kinds of soil, and in culture media without soil, in water or sand, containing various nutrients with different concentrations, when all the elements present in the culture are in an available form. In addition, it is possible that in the case of either deficiency or excess of a certain kind of nutrient, plants may not achieve normal growth, preventing the extraction of the necessary volume of xylem sap. Setting the assayable concentration range will also be necessary in the future.

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