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Effects of fullerene C_{60} on the uptake of nitrogen and mineral elements in crops using synchrotron radiation micro-X-ray fluorescence spectrometry (SR-μXRF) and stable isotope labelling†

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The unique characteristics of fullerene (C_{60}) have attracted great attention in the agricultural field. However, its potential effects on nitrogen sources and the uptake of various mineral nutrients required for plant growth remain unclear. In this study, we take advantage of the stable isotope ^{15}N labeling technique combined with synchrotron radiation micro-X-ray fluorescence spectrometry (SR-μXRF) to investigate efficiently the effects of C_{60} (70–200 nm) on the uptake level of nitrogen and multiple mineral elements in three common crops (maize, wheat, and soybean). The results showed that C_{60} had different effects on the uptake of nitrogen and 15 mineral elements in different types of crops. C_{60} significantly decreased the uptake rate of nitrate nitrogen in maize and soybean by 52.4% and 66.1%, respectively, but it had no significant effects on the uptake of ammonium nitrogen. In contrast, C_{60} had no significant effect on the uptake of nitrate nitrogen in wheat, but it significantly increased the uptake rate of ammonium nitrogen by more than 3-fold. In addition, C_{60} tended to change the uptake of 15 mineral elements in wheat, maize and soybean, but significant differences were found only in the uptake of K, Ca and Fe in different tissues of three crops. Our results suggest that the joint analysis technology not only facilitates the simultaneous comparison of the uptake of total mineral nutrients (including organic and inorganic nutrients) in plants but also enables us to obtain the impact of nanomaterials on plant growth. C_{60} can improve the uptake of nitrogen and change mineral elements in crops, possibly avoiding damage to soils and the environment caused by the overuse of fertilizers and increasing the yield quantity and quality of crops.

Environmental significance

Fullerenes and their derivatives, as potential nutritional protectants or trace nutrient liquid additives, can affect the absorption and transport of nitrogen and mineral nutrients in plants and have great prospects for increasing crop yield. This study investigates the effects of C_{60} on the uptake of nitrogen and 15 mineral elements in typical crops such as maize, wheat, and soybean using the stable isotope ^{15}N labelling technique and synchrotron radiation micro-X-ray fluorescence (SR-μXRF) technique. This innovative joint analysis technology can efficiently, non-destructively and simultaneously monitor and distinguish for changes in nitrogen sources and multiple mineral nutrients without any complex sample pretreatment, which will greatly accelerate the simultaneous fingerprints of trace or mineral nutrients in plants.

1. Introduction

Engineered nanomaterials, because of their unique chemical and physical properties such as high ratio of surface area to volume, extraordinary electronic and optical attributes, capability to engineer electron transfer, highly reactive surfaces, *etc.*, have been used in biomedical, electrical and industrial fields.[1–3] In particular, they have been also expanded to increase the quantity and quality of agricultural crop products.[4–6] Nanomaterials include carbon nanomaterials (CNMs) and non-carbon nanomaterials

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(nCNMs). Some reports indicate that the utilization of nCNMs, such as metal and metal oxide nanoparticles, can have detrimental effects on plants. For instance, the application of silver nanoparticles (AgNPs) exhibits toxicity towards plant growth[7,8] and leads to their release into the environment, causing ecological issues.[9] Furthermore, a considerable amount of research has shown that different metal nanomaterials have a negative impact on plant growth.[10–12] CNMs, composed of carbon atoms, exhibit excellent biocompatibility. They are not considered as environmental pollutants in aqueous solutions.[13] Moreover, the small size allows carbon nanomaterials to easily cross cell walls and membranes to enter plant cells. Specific CNMs possess the ability to impede plant uptake of organic pollutants,[14,15] enhance plant photosynthesis, and facilitate crop growth.[16] However, the widespread applications of these nanomaterials have triggered some concerns about their potential adverse effects on both the environment and human health. Worldwide, there have been numerous reports to obtain insight on new interactions of carbon nanomaterials with plants.[17–25] Published research reported positive or negative effects of CNMs on plant toxicity, biomass accumulation, development stages, nutritional and pharmaceutical compound accumulation and even some contradictory effects.[18,19] For example, C_{60} (0.09 mg L^{-1}, 98 nm) can reduce the contents of photosynthetic products and chlorophyll, eventually causing a sub-lethality in *Scenedesmus obliquus* and inhibiting the growth of *Lemna gibba* (1–10 mg L^{-1}, 29–38 nm).[20,21] Carboxylated C_{60} (0.01 mg mL^{-1}, 146 nm) can reduce cell viability and the number of mitochondria, impair cellular function, and increase the levels of intracellular reactive oxygen species, thereby inhibiting the growth of tobacco BY-2 suspension cells and *Arabidopsis* seedlings.[22] Multi-walled carbon nanotubes (MWCNTs) (0, 20, 200, 100, 2000 mg L^{-1} and 200 nm) can significantly inhibit the root and shoot lengths in lettuce, red spinach, cucumber and rice.[23] However, some studies report that carbon nanomaterials can promote the growth of plants. For example, MWCNTs (10–40 μg mL^{-1}) can significantly increase the germination rate and improve the growth of tomato seedlings.[24] Fullerenol $C_{60}(OH)_{20}$ (0.943, 4.72, 9.43, 10.88, 47.2 nM, 1.5 ± 0.2 nm and 5.0 ± 0.7 nm) can increase the water content, plant biomass and fruit yield in bitter melon (*Momordica charantia*).[25] Single-walled carbon nanotubes (28, 160, 900, 5000 mg L^{-1}, 8 nm) have no effects on the growth of cabbage and carrots but significantly enhance the elongation of roots in onion and cucumber.[26] Obviously, the small size of carbon nanomaterials is beneficial for their transmission into plant cells; however, the biological effects and potential impact of CNMs on crops is still challenging, which is not only related to the concentration of nanomaterials but also closely related to their shape, surface modification and the microenvironment of crops. A thorough understanding of the ecological effects of CNMs is required.

Fullerene (C_{60}), as one of the most typical carbon nanomaterials, has unique physical and chemical properties based on its unique cage structure and surface modified properties and has been applied for crop growth, soil remediation and environmental pollution control, *etc.* in the agricultural field.[15,27–30] The effects of C_{60} on plant growth have been reported. These studies focused on plant growth, physiological responses, and changes in biomolecular function. For example, Kumar *et al.* studied the effects of fullerenes on seed germination, and the results showed that fullerenes (<100 mg L^{-1}) did not affect seed germination, but at higher concentrations (200 mg L^{-1}) would cause toxicity to seedling growth.[31] Our previous studies showed that ^{13}C-labelled C_{60} (20 mg L^{-1}, 100 mg L^{-1}) could be absorbed by rice root and affected rice growth by reducing phytohormone levels in rice without significant concentration effect.[19] Stable isotope ^{13}C-labelled fullerenols (2.5, 5, 10 μg mL^{-1}, and 95 nm) can enter the roots of wheat, promote root elongation and enhance the synthesis of chlorophyll.[28] Avanasi *et al.* investigated the absorption and transport of ^{14}C-C_{60} (1.01 μg g^{-1}, ~1500 nm) in different plants and its biodegradation in different soils, and studied the absorption of C_{60} by radish through a hydroponic system. The results showed that C_{60} can be absorbed by plant roots and transported to underground parts of plants.[29] Although the particle size of C_{60} in the study was larger than 1500 nm, ^{14}C-labeled C_{60} could be detected in plant roots. De La Torre-Roche *et al.* studied the effect of fullerenes (500–5000 mg L^{-1}, 1450–1900 nm) on the immobilization of pesticide residues.[15] They found that pesticide accumulation varies greatly with crop species and carbon nanomaterial type/concentration. Zucchini and tomato growth was unaffected by carbon nanomaterial co-exposure, while C_{60} at 500 mg kg^{-1} reduced corn and soybean biomass. Meanwhile, the effect of C_{60} on the absorption of organic pollutants in plants has also been reported.[30,32] C_{60} nanoparticles (1670 mg kg^{-1}) had no impact on the biomass of plants and had little impact on weathered dichlorodiphenyldichloroethylene bioaccumulation in plants.[30] C_{60} nanoparticles (2–15 mg L^{-1}, ~50 nm) did not result in any acute toxicity to plants and increased plant uptake of trichloroethylene.[32] Then, for the impact of fullerenes on plants, although there are complex concentration effects, even if the particle size is relatively large and the concentration is suitable, it will not damage the growth of crops. However, the effects of C_{60} on the uptake of different nitrogen and mineral nutrients in crops have been seldom reported. The simple, real-time and non-destructive detection or evaluation methods of nitrogen nutrient elements and a variety of mineral nutrient elements are also full of challenges.

To grow and develop, plants need a variety of nutrients; nutrients are mainly absorbed through the root system and then transported to other parts of the plant. Mineral elements play an important role in the electron transport chain, the synthesis of biological macromolecules (enzymes, hormones, vitamins, nucleic acids, *etc.*), and other physiological and biochemical functions. The changes in the contents of mineral elements can remarkably influence the growth, development or reproduction, physiological processes in plants. Nitrogen is one of the essential elements for plant growth and development, and it is the main component of proteins, nucleic acids, chlorophyll, vitamins, alkaloids, and hormones in plants.[33–35] Nitrogen deficiency or excess has become an important factor limiting crop production and quality.[36] Plants can absorb and utilize several forms of nitrogen in soils, including ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and some organic nitrogen with low molecular weights.[37,38] However, many chemical products, such as pesticides, fertilizers and nutrient additives, have been widely used to increase crop yields. Their excessive use has disrupted the balance of soil minerals and reduced soil fertility, which substantially influences the contents of ammonia nitrogen, nitrate nitrogen and organic nitrogen in the soil and degraded the agricultural environment.[39,40] Appropriate application of nitrogen fertilizers is a key measure to achieve high crop yields and reduce environmental pollution.[41,42] Hao *et al.* found that carbon nanomaterials can increase the activities of some enzymes related to nitrogen metabolism in maize leaves and roots, thus promoting nitrogen utilization and plant growth.[43] Zhao *et al.* showed that the application of nano-carbon had a great promotion effect on the soil urease activity of different soil types.[44] Recently, studies have shown that nanomaterials play an important role in the absorption of mineral elements in plants. Abdel Latef *et al.* found that $nTiO_2$ (0.01%, 0.02%, 0.03%, and 25 nm) supplements significantly increased the activity of enzymatic antioxidants and the levels of soluble sugars, amino acids and proline in salt-affected plants.[45] Suriyaprabha *et al.* found that silica nanoparticles (15 g L^{-1}, 20–40 nm) can be absorbed by the roots of maize (*Zea mays* L.) and then migrate and accumulate in the leaves, which increase the total leaf protein content, improve the absorption of trace elements (such as copper, iron, manganese, and zinc) and finally improve the growth performance of maize.[46] Obviously, the uptake and transport (translocation, transformation) of nutrients in plants is of great significance for plant physiology and growth. In addition, fullerenes and their derivatives possess the potential ability to effectively permeate cell membranes and serve as carriers for both macro and trace elements.[47] Recently, G. G. Panova *et al.* reported the positive effects of water-soluble fullerene derivatives (1 mg kg^{-1}, 10 mg kg^{-1}, and 100 mg kg^{-1}) on the content of macro- and microelements in the soil and in plants (Chinese cabbage, tomato, and cucumber) physiological state, growth, and element content.[48] Fullerene derivatives could activate nitrogen transformation in the soil, enhance the process of nitrification, and promote the migration of some macro- and microelements (such as selenium and zinc elements) from soil to cucumber leaves at appropriate concentrations and improve the physiological state and growth of plants. They believe that fullerene and its derivatives, as a nanopreparation for soil or vegetation, a nutrient protectant, or an additive for trace nutrient element liquids, have good perspectives for improving crop production.[48,49] Therefore, it is necessary to study the effects of C_{60} on the acquisition and retention of nitrogen and mineral elements in typical crops. The study is expected to explore the potential agricultural application of C_{60} and to understand the role of C_{60} in regulating nutrient cycling in agroecosystems and improving crop yields.

Wheat, maize and soybean have different root systems; they are sensitive to changes in environmental conditions and pollutants, which enables them to be used as environmental and biological indicators for assessing the environmental safety of nanomaterials.[50] Plant roots have a large surface area to absorb mineral nutrients (inorganic ions) from the soil. After absorption on the roots, mineral nutrients are transported to other parts of the plant, where they are utilized to perform various biological functions. In this study, we investigated the effects of C_{60} on the uptake of nitrogen and 15 mineral elements in three crops using the stable isotope ^{15}N labelling technique and synchrotron radiation micro-X-ray fluorescence (SR-μXRF) technique and attempted to unravel the underlying mechanisms. The stable isotope labelling technique is a simple but powerful method with high accuracy, low detection limit, nonradioactive nature, high stability, and suitability in long-term tracing, which have been used to quantify and trace the biological behaviors of nanomaterials in complex biota and ecological environments.[28,51–54] Mineral nutrient elements in plants are detected by conventional analysis technology, such as atomic absorption spectrometry, atomic fluorescence spectrometry, inductively coupled plasma mass spectrometry, *etc.*, which often have the disadvantages of complex sample process, long time, large amounts of samples, poor reproducibility and so on. The SR-μXRF technology provides an important tool for monitoring the multi-elemental distribution in the microscopic tissue of organisms at low levels, which has the advantages of high sensitivity, real-time, non-destructive, and simultaneous analysis of multiple elements.[55] We processed plants in a simple way and simultaneously obtained the distribution information of multiple mineral nutrients in three crops by the SR-μXRF technology. Combined with isotope labelling technology, we distinguished three forms of nitrogen sources and intuitively obtained the influence of C_{60} on the nitrogen absorption level of different types of crops. This combined analysis technique allows us to quickly and efficiently study the effects of CNMs on nitrogen and mineral element uptake in plants. The study is expected to provide new ideas for the agricultural application of C_{60} to regulate crop nutrients and promote crop productivity.

2. Materials and methods

2.1 Materials and reagents

Fullerene C_{60} (99%) was purchased from Suzhou Dade Carbon Nanotechnology Co., Ltd (Suzhou, China). Seeds of soybean, maize and wheat were purchased from the Institute of Crop Science, Chinese Academy of Agricultural Sciences

(Beijing, China). Ammonium sulfate and potassium nitrate, with or without ^{15}N labeling, were purchased from Shanghai Maclin Biochemical Technology Co., Ltd. (Shanghai, China). Soils were taken from a farmland in the suburb of Beijing (116.23″E, 39.99″N). XRF tape (TF-500) was purchased from DHJ Analysis Co., Ltd. (Beijing, China). The chemicals and reagents used in this study were of analytical grade or chromatographic grade.

2.2 Characterization of C_{60}

The morphology and size of C_{60} in water were characterized using a scanning electron microscope (S-4800, Hitachi, Tokyo, Japan) and a nanosizer (Zetasizer Nano ZS90, Malvern, UK), respectively. To measure the size, C_{60} was dispersed in water at a concentration of 0.2 mg mL^{-1} and then sonicated for 5 min to make it disperse well in water. The size distribution was determined using the Zetasizer Nano ZS90, and a small amount of C_{60} aqueous solution was dropped on the silicon wafer and given surface spray gold treatment, and then observed by scanning electron microscopy. Furthermore, the cage-like carbon molecular structure of C_{60} was characterized using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS, Autoflex III, Bruker, Germany). To avoid the interference of matrix, no matrix was used here. In addition, to increase the signal-to-noise ratio, C_{60} was directly dispersed in toluene, deposited on a grid and dried at room temperature. Toluene was used here as solvent because it can not only increase the solubility of C_{60} but also greatly reduce the diffusion of C_{60} on the smooth metal surface due to its high volatility, thereby ensuring the concentration and enrichment of C_{60} on the target and improving the signal-to-noise ratio.

2.3 Experimental

2.3.1 C_{60} treatment. C_{60} (500 mg) was mixed with 50 g of air-dried soils and placed in a 50 mL large plastic centrifuge tube. The concentration of C_{60} was 10.0 mg g^{-1}. Soil without C_{60} was used as the control. The seeds of maize, wheat, and soybean were germinated. Healthy seedlings were selected and planted in centrifuge tubes, with buds facing up and roots facing down, 4 replicates per group (a total of 48 tubes). Seedlings were irrigated with water at soil water-holding capacity of 70% and grown under a photoperiod of 12 h light/12 h dark at 25/20 °C day/night and 80% humidity for 30 days. After treatment, seedlings with their roots were collected and rinsed with water 3–5 times. The plants were labelled with ^{15}N as described in section 2.3.2. After labelling, roots, stems and leaves were separated, and their fresh weights were measured. Then, they were lyophilized for 48 h to constant weight, and their dry weights were measured. Finally, the dried roots, stems and leaves were ground into powder, which was subject to synchrotron radiation micro-X-ray fluorescence analysis to observe the changes in the contents of mineral elements.

2.3.2 Stable isotope ^{15}N labelling. Two forms of nitrogen, including ammonia nitrogen and nitrate nitrogen, were labeled with stable isotope ^{15}N. Solutions of ammonium sulfate and potassium nitrate, with or without ^{15}N labelling, were separately prepared at a nitrogen content of 100 μmol N per L. A 100 μmol N per L concentration level for nitrogen is employed to simulate plant uptake under high-nitrogen conditions.[56] The higher concentration level enables us to better observe and compare plants' nitrogen absorption levels when exposed to different nitrogen sources within a high-nitrogen background. To prevent potential transformation of nitrogen and maintain the membrane stability of root cells, the solution was supplemented with penicillin (10 mg L^{-1}) and $CaCl_2{\cdot}2H_2O$ (100 μmol L^{-1}). After 30 days of cultivation as described above, the plants with the roots were collected and washed. The roots were placed in 15 mL centrifuge tubes containing 100 μmol N per L ammonium sulfate and potassium nitrate, with or without ^{15}N labeling, and incubated for 4 h. After incubation, roots, stems and leaves were separated and washed with 50 mmol L^{-1} KCl and distilled water. They were dried in an oven at 70 °C for 48 h, weighed, and ground to powder in a ball mill. The nitrogen content and the ratio of isotope (^{15}N/^{14}N) were determined using a MAT 253 stable ratio mass spectrometer (Thermo Fisher, USA) coupled with a Flash 2000 HT elemental analyzer (Thermo Fisher, USA). The uptake rate of nitrogen was calculated using the following equation (eqn (1)).

$$N\ (\mu\text{g N per g d.w. tissue per h}) = \frac{\text{APE}_{\text{sample}} \times \text{d.w. (g)} \times N_{\text{content}}\ (\%)}{\text{APE}_{\text{added}} \times \text{d.w. (g)} \times t\ (\text{h})} \tag{1}$$

where N (μg N per g d.w. root per h) is the nitrogen absorption rate, which indicates the contents of nitrogen absorbed per gram of dry weight tissue per hour, $\text{APE}_{\text{sample}}$ is the atom percent excess of ^{15}N in plant tissues, which is calculated by subtracting that of ^{15}N in control plant tissues from the percentage of ^{15}N in labelled plant tissues, d.w. refers to the dry weight of plant tissue, and N_{content} refers to the percentage content of nitrogen in plant tissues. $\text{APE}_{\text{added}}$ is the atom percent excess of ^{15}N in the added nitrogen source, which is calculated by subtracting that of ^{15}N in the atmosphere from the percentage of ^{15}N in the added nitrogen source, and t refers to the labelling time.

2.3.3 Measurement of mineral elements using SR-μXRF. The pressed pellets (6 mm in diameter and 30–100 μm in thickness) of dried tissue powders (about 20.0 mg) were prepared using a PP-20S automatic powder tablet press machine (Tianjin Jingtuo Instrument, Tianjin, China) under about 700 MPa for 30 s. The pellets were analyzed using SR-μXRF with an electron beam energy of 2.5 GeV, a current intensity of up to 120 mA and an incident beam slit of 50 μm × 50 μm. A silicon–lithium semiconductor detector (type Link-ISIS) was used to obtain the spectra, with the sample at 45° to the detector and at 90° to the incident beam. The distance between the beryllium window and the sample was

80 mm. The spectra were recorded and analyzed using PyMca, with a spectral collection time of 10 s. On each sample, a total of 70 points were randomly selected and measured to obtain the raw data of trace elements (Ar, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Ga, As, Se, Kr, and Br) in plant tissues.

2.4 Data analysis

The data of samples were processed using PyMca 5.5.5. After selecting mineral elements of interest, the fit area values of the *K* energy layer were normalized due to the variations in measurement conditions (such as different incident light intensities, different measurement times, and different geometric structures). The experiment was repeated in triplicate. Data were processed in Excel, and figures were generated using Origin 2020 (OriginLab, Massachusetts, USA). Data are expressed as mean ± SD ($n = 3$).

3. Results and discussion

3.1 Characterization of C_{60}

The absorption of nanomaterials by plants is affected by the particle size, shape, exposure conditions and concentration of nanomaterials, and the particle size is one of the main reasons affecting plant absorption. Slomberg *et al.* reported that SiO_2 nanomaterials up to 200 nm could be absorbed by the roots of *Arabidopsis thaliana*.[57] Larue *et al.* investigated in detail the accumulation of TiO_2 with different particle sizes (36–140 nm) in wheat (*Triticum aestivum*). They found that the smaller the particle size (<36 nm), the easier to be accumulated in the root system and distributed throughout the plant tissue without dissolution or transformation, while nanoparticles of 36–140 nm accumulated in the root substance of wheat but did not transfer to the branches, and nanoparticles >140 nm did not accumulate in the root system of wheat.[58] The size of nanoparticles influences their ability to enter plant cells, affecting their transport and crop growth.[59] For carbon-based nanomaterials, due to their unique nano characteristics and exposure environment, from several nanometers to several micrometers, they may enter the plant roots and affect the physiological functions and growth of plants. Thus, mentioning particle size is essential for a comprehensive understanding of the effects of C_{60} nanomaterials on plants. Scanning electron microscopy (SEM) images (Fig. 1A) showed that C_{60} could form very small aggregates in water, with diameters ranging from 70 to 200 nm. As shown in Fig. 1B, the hydrated diameters of C_{60} in water were measured as around 131 nm by dynamic light scattering (DLS) measurement, which had a lognormal hydrated particle size distribution, mainly ranging from 68 to 220 nm. C_{60} nanoparticles in this size range may be absorbed by plant roots and transported to the aboveground part of the plant, affecting the potential internal physiology of the plant. Besides, most of the large size C_{60} nanoparticles may also remain outside the roots and enriched in the soil, influencing the availability of mineral elements in the soil,[48] which in turn may affect the absorption and transport of these mineral elements in plants. Additionally, there was a single characteristic peak on the MALDI-TOF-MS spectrum of C_{60} at $m/z = 719.59$ (Fig. 1C), which is very close to the theoretical m/z of 720 for C_{60} and is consistent with other literature.[60–62]

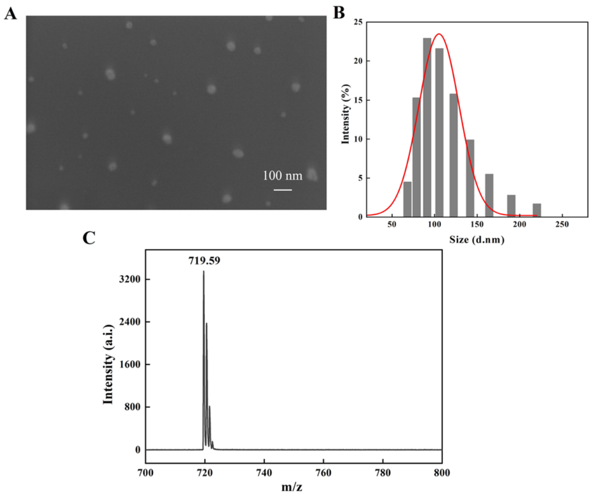

Fig. 1 Characterization of C_{60}. (A) SEM image, (B) DLS image, (C) time-of-flight mass spectrum.

3.2 Effects of C_{60} on the biomass of three crops

C_{60} affected the biomass of three crops (maize, soybean and wheat) to a certain extent, but had no significant change or

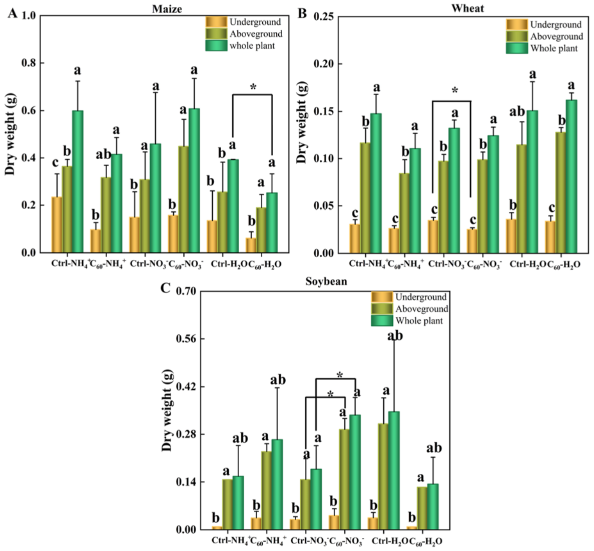

Fig. 2 Effects of C_{60} on the biomass of three crops. (A) Maize, (B) wheat, and (C) soybean. Data are expressed as mean ± SD ($n = 4$). Differences were considered statistically significant at $P < 0.05$. Ctrl: control. The lowercase letters (a–c) represent statistically significant differences between variables. * indicates significant difference between the control and the C_{60} treatment.

toxic effects (Fig. 2 and S1 and Tables S1–S3 in the ESI†). In both control and C_{60} treatment groups, the crops were cultivated under the same conditions for 30 days, and to study the effects of C_{60} on the uptake of different forms of N, they were separated from the soils and incubated in water containing two forms of N (ammonia N and nitrate N) with or without ^{15}N labelling for 4 h. The addition of nitrogen tended to promote the growth of maize compared with the water control group, but no significant changes in dry weights were found. Our results showed that C_{60} did not remarkably improve the growth of three crops, only with significant changes in dry weights found in individual groups. As for maize (Fig. 2A), the dry weight of the whole plant in control-H_2O was significantly higher than that in C_{60}-H_2O, and there was no significant difference in dry weights of the whole plant, underground (roots), and aboveground (stem–leaves) parts of the plant between other groups. As for wheat (Fig. 2B), the dry weight of underground parts in C_{60}-NO_3^- decreased significantly compared to that in control-NO_3^-, while there was no significant difference in other groups. As for soybean (Fig. 2C), the dry weights of aboveground parts (stem–leaves) of the plant and the whole plant in C_{60}-NO_3^- increased significantly compared to that in control-NO_3^-, while there was no significant difference in other groups. Therefore, the inhibitory effects of high concentrations of C_{60} on crop growth could be ignored, and the effects of C_{60} on the uptake of N and mineral elements could be clearly determined.

3.3 Effects of C_{60} on the uptake of N element by three crops

Maize, wheat and soybean had different uptake rates of nitrogen: soybean (ammonia-N 150.41 ± 10.01, nitrate-N 71.19 ± 21.25 μg N per g d.w. tissue per h) > maize (ammonia-N 36.26 ± 9.7, nitrate-N 78.71 ± 22.11 μg N per g d.w. tissue per h) > wheat (ammonia-N 12.39 ± 4.02, nitrate-N 31.95 ± 4.42 μg N per g d.w. per tissue h). Soybean has an intrinsic nitrogen fixation ability, and its root cavities are similar in size to those in maize. In both soybean and maize, the nitrogen uptake capacity of the underground parts (roots) is about an order of magnitude higher than that of stems and the aboveground parts (stem–leaves). In contrast, the root cavities of wheat are relatively small, and the nitrogen uptake capacity of underground is lower than that of aboveground parts, indicating that wheat has the lowest nitrogen uptake capacity among these three crops. As shown in Fig. 3 and Tables S4–S6 in the ESI,† C_{60} had different effects on the uptake rates of two forms of N in these three crops. In untreated maize (Fig. 3A), the NO_3^--N uptake rate was higher than the NH_4^+-N uptake rate, and the N uptake rate of underground was significantly higher than that of aboveground parts of maize. After C_{60} treatment, the NO_3^--N uptake rate significantly decreased from 78.71 ± 22.11 μg N per g d.w. tissue per h to 37.49 ± 7.39 μg N per g d.w. tissue per h, but C_{60} had no significant effects on the NH_4^+-N uptake rates. As for the untreated wheat (Fig. 3B), the NO_3^--N uptake rate was higher than the NH_4^+-N uptake rate, and the NO_3^--N uptake rate of aboveground pats was also significantly higher than the NH_4^+-N uptake rate of aboveground parts of wheat. After C_{60} treatment, the NH_4^+-N uptake rate significantly increased from 12.39 ± 4.02 μg N per g d.w. tissue per h to 50.92 ± 16.38 μg N per g d.w. tissue per h, but there were no significant changes in the NO_3^--N uptake rates. In untreated soybean (Fig. 3C), the NH_4^+-N uptake rate was higher than the NO_3^--N uptake rate, and the N uptake rate of underground was significantly higher than that of aboveground parts. After C_{60} treatment, the NO_3^--N uptake rate significantly decreased from 71.19 ± 21.25 μg N per g d.w. tissue per h to 24.13 ± 8 μg N per g d.w. tissue per h, but there were no significant changes in the NH_4^+-N uptake rates.

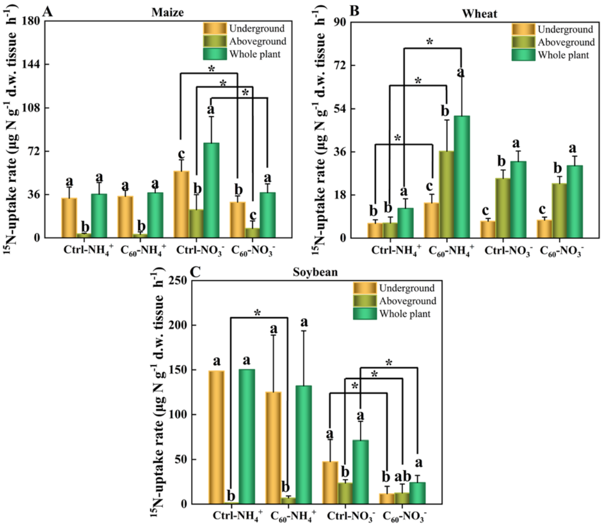

Fig. 3 Effects of C_{60} on the nitrogen uptake rate of different crops. (A) Maize, (B) wheat, and (C) soybean. Data are expressed as mean ± SD (n = 4). Differences were considered statistically significant at $P < 0.05$. Ctrl: control. The lowercase letters (a–c) represent statistically significant differences between variables. * indicates significant difference between the control and the C_{60} treatment.

Here, our results showed that C_{60} could affect the uptake of nitrogen in three crops depending on crop types and nitrogen forms. In maize and soybean, C_{60} could significantly reduce the uptake of nitrate-N. In wheat, however, C_{60} could significantly increase the uptake of ammonia-N. This result may be because C_{60} interferes with the activity of nitrate reductase, glutamate dehydrogenase and glutamine synthetase,[63] thus converting inorganic nitrogen into organic nitrogen, resulting in the conversion and utilization of nitrate nitrogen and ammonia nitrogen in plants. The investigation showed that C_{60} could regulate the ability of crops to absorb different forms of nitrogen, which was expected to optimize the application of nitrogen fertilizer in different crops.

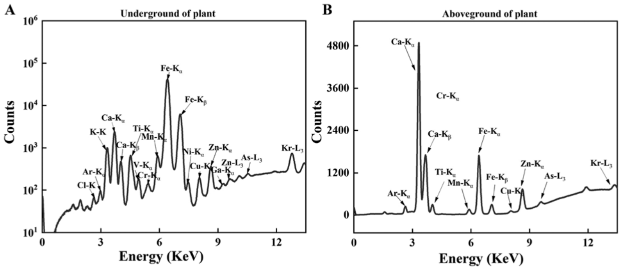

Fig. 4 The SR-μXRF spectrum showing the presence of mineral elements in the (A) underground and (B) aboveground parts of plant.

3.4 Effects of C_{60} on the uptake of mineral elements by three crops

We measured the levels of 15 mineral elements in the tissues of three crops using SR-μXRF. As shown in Fig. 4, the SR-μXRF spectrum clearly showed the presence of 15 mineral elements in the underground and aboveground parts of plants. In three crops, the uptake levels of mineral elements in their underground were higher than the aboveground parts (Fig. 5 and 6). C_{60} treatment had different impacts on the uptake levels of mineral elements in different crops. In maize, C_{60} had little effects on the uptake levels of most of the mineral elements in the underground and aboveground parts of plant, but it caused a significant increase in the uptake level of Fe in the aboveground parts of plant (Fig. 6). In wheat, except for a significant decrease in the uptake level of Fe in the underground parts (Fig. 5) and an increase in the uptake level of K in the aboveground parts (Fig. 6), C_{60} had almost no effect on the uptake levels of the other mineral elements in the plants. Meanwhile, in soybean, C_{60} had no significant effects on 15 mineral elements in the underground parts of the plant. On the other hand, C_{60} significantly increased the level of Fe but significantly decreased the levels of Ca in the aboveground parts of soybean, with no effects on other mineral elements. The results suggested that C_{60} could change the uptake of mineral elements in the underground but exhibited differential effects on the uptake of mineral elements in aboveground parts. These results suggested that exposure to a certain concentration of fullerenes in soil can affect the absorption and transport of useful mineral elements (such as K, Ca, and Fe) by plants. Panova *et al.* had confirmed that the use of fullerenes and their derivatives will allow mineral elements to enter the plant, affecting the metabolic process and photosynthesis of the plant.[49] Additionally, due to the increase of relevant mineral elements in the plant, the osmotic pressure in its cells may lead to more efficient water transport, thus affecting the plant.[48] Moreover, C_{60} had more significant effects on the uptake of N and mineral elements in soybean than that of maize and wheat, possibly because soybean processes an intrinsic nitrogen fixation ability and has a deeper and more vertically oriented root system.[64] It is reported that nanomaterials enter the plants mainly through the roots,[65] so C_{60} had a more profound effect on the uptake of N and mineral elements in soybean.

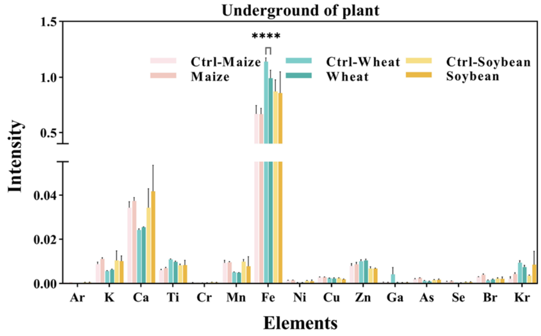

Fig. 5 Effects of C_{60} on the levels of mineral elements in the underground parts of maize, wheat and soybean plants. * indicates significant difference between the control and the C_{60} treatment at $P < 0.05$.

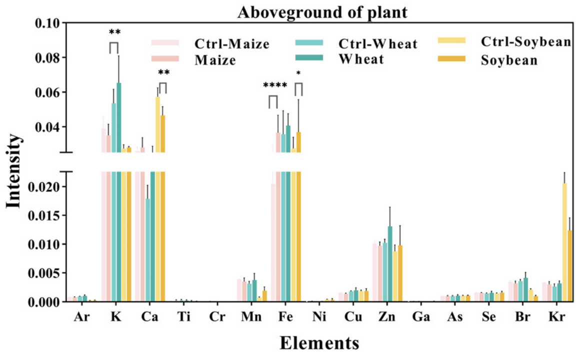

Fig. 6 Effects of C_{60} on the levels of mineral elements in the aboveground parts of maize, wheat and soybean plants. * indicates significant difference between the control and the C_{60} treatment at $P < 0.05$.

Conclusions

For the uptake level of nitrogen and multiple mineral elements in plants, the advantage of the stable isotope labelling technique combined with synchrotron radiation micro-X-ray fluorescence spectrometry (SR-μXRF) can efficiently and non-destructively provide test data for almost all changes in element content without any complex sample pretreatment. Although C_{60} did not significantly improve the growth and invoke physiological responses in maize, wheat and soybean, it could regulate the uptake of nitrogen and mineral elements by these crops. The effects of C_{60} on the uptake of nitrogen and mineral elements differed in different crops, even in their tissues (roots and stems–leaves). In maize and soybean, C_{60} could significantly reduce the uptake of nitrate-N. In wheat, however, C_{60} could significantly increase the uptake of ammonia-N. In addition, C_{60} had little effect on the uptake of mineral elements in maize and wheat, but it could remarkably affect the uptake of some mineral elements in soybean. Our results demonstrated that C_{60} had great potential to be used as a fertilizer to improve the use efficiency of nitrogen fertilizers and mineral element fertilizers in agriculture. Simultaneously, we have also explored an analytical method that utilizes stable isotope labelling technology combined with synchrotron radiation micro-X-ray fluorescence spectrometry for comprehensive testing of the changes of nutrient elements in plants, which will greatly accelerate the fingerprint spectrum analysis of all of the microscale or mineral nutrient elements in plants. Accordingly, this research offers novel insights into utilizing fullerenes for regulating crop nutrients to improve productivity while also presenting prospects for applying nano-carbon materials in agricultural production.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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